PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 11 September 2000 (11.09.00)	
International application No. PCT/US99/25433	**Applicant's or agent's file reference** UTFG:240P
International filing date (day/month/year) 29 October 1999 (29.10.99)	**Priority date** (day/month/year) 29 October 1998 (29.10.98)
Applicant COPLAND, John, A., III et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

23 May 2000 (23.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Charlotte ENGER
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

WIM-SHS-STN

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To: MARK B. WILSON
FULBRIGHT & JAWORSKI, L.L.P.
600 CONGRESS AVENUE, SUITE 2400
AUSTIN, TEXAS 78701

RECEIVED
F & J
AUSTIN INTL
OCT 26 2000

PCT

NOTIFICATION OF TRANSMITTAL OF INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of Mailing *(day/month/year)*	21 SEP 2000

Applicant's or agent's file reference	IMPORTANT NOTIFICATION
UTFG:240P	

International application No.	International filing date *(day/month/year)*	Priority Date *(day/month/year)*
PCT/US99/25433	29 OCTOBER 1999	29 OCTOBER 1998

Applicant
BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices)(Article 39(1))(see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

FULBRIGHT & JAWORSKI LLP
AUSTIN, TEXAS

DOCKETED
DATE 1 PER R.C.'S
INITIALS CLC

SEP 26 2000

RECEIVED

Name and mailing address of the IPEA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	FREDERICK KRASS Dorothea Lawrence for
Facsimile No. (703) 305-3230	Telephone No. (703) 308-1235

Form PCT/IPEA/416 (July 1992)★

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference UTFG:240P	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/25433	International filing date *(day/month/year)* 29 OCTOBER 1999	Priority date *(day/month/year)* 29 OCTOBER 1998
International Patent Classification (IPC) or national classification and IPC IPC(7): A61K 31/427 and US Cl.: 514/369		
Applicant BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 3 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
23 MAY 2000	06 SEPTEMBER 2000
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Dorothea Laurence Tor FREDERICK KRASS Telephone No. (703) 308-1235

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

[redacted]tional application No.
PCT/US99/25433

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-33 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the claims:
pages 34-36 , as originally filed
pages NONE , as amended (together with any statement) under Article 19
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the drawings:
pages 1-6 , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE , as originally filed
pages NONE , filed with the demand
pages NONE , filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

[redacted]ational application No.
PCT/US99/25433

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	1-21	YES
	Claims	NONE	NO
Inventive Step (IS)	Claims	1-21	YES
	Claims	NONE	NO
Industrial Applicability (IA)	Claims	1-21	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-21 meet the criteria set out in PCT Article 33(2)-(3), because the prior art does not teach or fairly suggest methods for preventing preterm labor by administering a thiazolidinedione to an individual in need thereof, nor methods for screening for appropriate compounds to use.

Claims 1-21 meet the criteria set out in PCT Article 33(4); the applicability of the claimed methods to the medical/pharmaceutical industries is self-evident.

---------- NEW CITATIONS ----------

NONE

PCT
REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference *(if desired) (12 characters maximum)* UTFG:240P

Box No. I TITLE OF INVENTION

CLINICAL USE OF THIAZOLIDINEDIONES ALONE OR IN CONJUNCTION WITH OTHER AGENTS TO BLOCK OXYTOCIN MEDIATED ACTIONS SUCH AS UTERINE CONTRACTIONS IN PREMATURE LABOR OR LACTATION

Box No. II APPLICANT

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM
201 WEST SEVENTH STREET
AUSTIN, TX 78701
US

☐ This person is also inventor.

Telephone No.

Facsimile No.

Teleprinter No.

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☒ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

COPLAND, JOHN A. III

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf of the applicant(s) before the competent International Authorities as: ☒ agent ☐ common representative

Name and address: *(Family name followed by give name; for a legal entity, full official designation. The address must include postal code and name of country.)*

WILSON, MARK B.
ARNOLD WHITE & DURKEE
750 Bering Drive
Houston, TX 77057-2198
United States of America

Telephone No. (713) 787-1400

Facsimile No. 713-787-1440

Teleprinter No.

☐ **Address for correspondence:** Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet is not to be included in the request.

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

IVES, KIRK L.

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

SOLOFF, MELVYN

This person is:
☐ applicant only
☒ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality: US

State *(that is, country)* of residence: US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated Sates except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: *(Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)*

This person is:
☐ applicant only
☐ applicant and inventor
☐ inventor only *(If this check-box is marked, do not fill in below.)*

State *(that is, country)* of nationality:

State *(that is, country)* of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) *(mark the applicable check-boxes; at least one must be marked):*

Regional Patent

☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SL Sierre Leone, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT

☒ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT

☒ **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT

☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, GW Guinea-Bissau, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT *(if other kind of protection or treatment desired, specify on dotted line)* .....

National Patent *(if other kind of protection or treatment desired, specify on dotted line):*

☒AE United Arab Emirates
☒AL Albania
☒AM Armenia
☒AT Austria
☒AU Australia
☒AZ Azerbaijan
☒BA Bosnia and Herzegovina
☒BB Barbados
☒BG Bulgaria
☒BR Brazil
☒BY Belarus
☒CA Canada
☒CH and LI Switzerland and Liechtenstein
☒CN China
☒CR Costa Rica
☒CU Cuba
☒CZ Czech Republic
☒DE Germany
☒DK Denmark
☒DM Dominica
☒EE Estonia
☒ES Spain
☒FI Finland
☒GB United Kingdom
☒GE Georgia
☒GD Grenada
☒GH Ghana
☒GM Gambia
☒HR Croatia
☒HU Hungary
☒ID Indonesia
☒IL Israel
☒IN India
☒IS Iceland
☒JP Japan
☒KE Kenya
☒KG Kyrgyzstan
☒KP Democratic People's Republic of Korea
☒KR Republic of Korea
☒KZ Kazakstan
☒LC Saint Lucia
☒LK Sri Lanka
☒LR Liberia
☒LS Lesotho
☒LT Lithuania
☒LU Luxembourg
☒LV Latvia
☒MA Morocco
☒MD Republic of Moldova
☒MG Madagascar
☒MK The former Yugoslav Republic of Macedonia
☒MN Mongolia
☒MW Malawi
☒MX Mexico
☒NO Norway
☒NZ New Zealand
☒PL Poland
☒PT Portugal
☒RO Romania
☒RU Russian Federation
☒SD Sudan
☒SE Sweden
☒SG Singapore
☒SI Slovenia
☒SK Slovakia
☒SL Sierra Leone
☒TJ Tajikistan
☒TM Turkmenistan
☒TR Turkey
☒TT Trinidad and Tobago
☒TZ The United Republic of Tanzania
☒UA Ukraine
☒UG Uganda
☒US United States of America (CIP)
☒UZ Uzbekistan
☒VN Viet Nam
☒YU Yugoslavia
☒ZA South Africa
☒ZW Zimbabwe

Check-boxes reserved for designating States (for the purposes of a national patent) which have become party to the PCT after issuance of this sheet:
☐

Precautionary Designation Statement: In addition to the designations made above, the applicant also makes under Rule 4.9(b) all other designations which would be permitted under the PCT except the designations(s) indicated in the Supplemental Box as being excluded from the scope of this statement. The applicant declares that those additional designations are subject to confirmation and that any designation which is not confirmed before the expiration of 15 months from the priority date is to be regarded as withdrawn by the applicant at the expiration of that time limit. *(Confirmation of a designation consists of the filing of a notice specifying that designation and the payment of the designation and confirmation fees. Confirmation must reach the receiving Office within the 15-month time limit.)*

Supplemental Box ***If the Supplemental Box is not used, this sheet should not be included in the request.***

1. If, in any of the Boxes, the space is insufficient to furnish all the information: in such case, write "Continuation of Box No. ..." [indicate the number of the Box] and furnish the information in the same manner as required according to the captions of the Box in which the space was insufficient, in particular:

(i) if more than two persons are involved as applicants and/or inventors and no "continuation sheet" is available: in such case, write "Continuation of Box No. III" and indicate for each additional person the same type of information as required in Box No. III. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below;

(ii) if, in Box No. II or in any of the sub-boxes of Box No. III, the indication "the States indicated in the Supplemental Box" is checked: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the applicant(s) involved and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is applicant;

(iii) if, in Box No. II or in any of the sub-boxes of Box No. III, the inventor or the inventor/applicant is not inventor for the purposes of all designated States or for the purposes of the United States of America: in such case, write "Continuation of Box No. II" or "Continuation of Box No. III" or "Continuation of Boxes No. II and No. III" (as the case may be), indicate the name of the inventor(s) and, next to (each) such name, the State(s) (and/or, where applicable, ARIPO, Eurasian, European or OAPI patent) for the purposes of which the named person is inventor;

(iv) if, in addition to the agent(s) indicated in Box No. IV, there are further agents: in such case, write "Continuation of Box No. IV" and indicate for each further agent the same type of information as required in Box No. IV;

(v) if, in Box No. V, the name of any State (or OAPI) is accompanied by the indication "patent of addition," or "certificate of addition," or if, in Box No. V, the name of the United States of America is accompanied by an indication "continuation" or "continuation-in-part": in such case, write "Continuation of Box V" and the name of each State involved (or OAPI), and after the name of each such State (or OAPI), the number of the parent title or parent application and the date of grant of the parent title or filing of the parent application;

(vi) if, in Box No. VI, there are more than three earlier applications whose priority is claimed: in such case, write "Continuation of Box No. VI" and indicate for each additional earlier application the same type of information as required in Box No. VI;

(vii) if, in Box No. VI, the earlier application is an ARIPO application: in such case, write "Continuation of Box VI", specify the number of the item corresponding to that earlier application and indicate at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed.

2. If, with regard to the precautionary designation statement contained in Box No. V, the applicant wishes to exclude any State(s) from the scope of that statment: in such case, write "Designation(s) excluded from precautionary designation statement" and indicate the name or two-letter code of each State so excluded.

3. If the applicant claims, in respect of any designated Office, the benefits, of provisions of the national law concerning non-prejudicial disclosures or exceptions to lack of novelty: in such case, write "Statement concerning non-prejudicial disclosures or exceptions to lack of novelty: and furnish that statement below.

CONTINUATION OF BOX V: US 60/106.133 FILED 29.10.98 (29 OCTOBER 1998)

Box No. VI PRIORITY CLAIM ☐ Further priority claims are indicated in the Supplemental Box

Filing Date of earlier application (day/month/year)	Number of earlier application	Where earlier application is: national application: country	Regional application:* regional Office	international application: receiving Office
item (1) 29.10.98 (29 OCTOBER 1998)	60/106.133	US		
item (2)				
item (3)				

☒ The receiving Office is requested to prepare and transmit to the International Bureau a certified copy of the earlier application(s) *(only if the earlier application was filed with the Office which for the purposes of the present international application is the receiving Office)* identified above as item(s): 1

* *Where the earlier application is an ARIPO application, it is mandatory to indicate in the Supplemental Box at least one country party to the Paris Convention for the Protection of Industrial Property for which that earlier application was filed (Rule 4.10(b)(ii)). See Supplemental Box.*

BOX No. VII INTERNATIONAL SEARCHING AUTHORITY

Choice of International Searching Authority (ISA) *(If two or more International Searching Authorities are competent to carry out the international search, indicate the Authority chosen; the two-letter code may be used):*

ISA EPO

Request to use results of earlier search; reference to that search (if an earlier search has been carried out by or requested from the International Searching Authority):
Date (day/month/year) Number Country (or regional Office):

BOX No. VIII CHECK LIST; LANGUAGE OF FILING

This international application contains the following **number of sheets:**

request	: 5	sheets
description (excluding sequence lissting part)	: 33	sheets
claims	: 3	sheets
abstract	: 1	sheets
drawings	: 6	sheets
sequence listing part of description	: 0	sheets
Total number of sheets:	48	sheets

This international application is **accompanied by** the item(s) marked below:

1. ☒ fee calculation sheet
2. ☐ separate signed power of attorney
3. ☐ copy of general power of attorney; reference number, if any:
4. ☐ statement explaining lack of signature
5. ☐ priority document(s) *identified in Box No. VI as item(s):*
6. ☐ translation of international application into (language):
7. ☐ separate indications concerning deposited microorganisms or other biological material
8. ☐ nucleotide and/or amino acid sequence listing in computer readable form
9. ☒ other (specify): **post card**

Figure of the drawings which should accompany the abstract:

Language of filing of the international application: ENGLISH

Box No. IX SIGNATURE OF APPLICANT OR AGENT

Next to each signature, indicate the name of the person signing and the capacity in which the person signs (if such capacity is not obvious from reading the request)

MARK B. WILSON, Applicant's Agent

October 29, 1999
Date

For receiving Office use only

1. Date of actual receipt of the purported international application:	2. Drawings:
3. Corrected date of actual receipt due to later but timely received papers or drawings completing the purported international application:	[ ] received:
4. Date of timely receipt of the required corrections under PCT Article 11(2):	[ ] not received
5. International Searching Authority (if two or more are competent): ISA/	6. [ ] Transmittal of search copy delayed until search fee is paid

For International Bureau use only

Date of receipt of the record copy by the International Bureau:

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
UTFG:240P		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 99/25433	29/10/1999	29/10/1998
Applicant		
THE UNIVERSITY OF TEXAS SYSTEM BOARD OF RE...et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [X] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [ ] the text is approved as submitted by the applicant.

   [X] the text has been established by this Authority to read as follows:

   USE OF THIAZOLIDINEDIONES DERIVATIVES FOR PREVENTING UTERINE CONTRACTIONS IN PREMATURE LABOUR OR LACTATION

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [ ] None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/25433

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-7, 10-19 relate to an rather elevated number of possible compounds (thiazolidinediones). Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Furthermore, the expression "a compound related to troglitazone" is not clear, because it is not known how such relation should be interpreted in structural terms.

Present claims 15,16 relate to compounds defined by reference to a desirable characteristic or property, namely the activity as a tocolytic agent, as a beta-mimetic, as prostaglandin inhibitor or as a calcium blocking agent.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the thiazolidindiones mentioned in claims 8,9, and to the compounds mentioned in claims 17-18, with due regard to the general idea underlying the present invention.

Claims searched completely: 8.
Claims searched incompletely: 1-7, 9-21

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched: This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

International Application No
[redacted]/US 99/25433

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/427 A61K31/4439 A61P15/06 A61K31/426 G01N33/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 39006 A (GREEN ALLAN ;UNIV TEXAS (US); URBAN RANDALL J (US)) 11 September 1998 (1998-09-11) abstract examples 1-8 claims 1-24 page 4, line 1 - line 10 ---	1-21
A	REECE E.A. ET AL: "Diabetes mellitus in pregnancy: What are the best treatment options?." DRUG SAFETY, (1998) 18/3 (209-220). , XP000889644 page 210, column 2, paragraph 2 page 215, column 2, paragraph 3 --- -/--	1-4,7,8, 10-14

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2000	10/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Bonzano, C

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 861 666 A (TAKEDA CHEMICAL INDUSTRIES LTD) 2 September 1998 (1998-09-02) page 2, line 10 - line 33 ---	1-21
A	EP 0 783 888 A (SANKYO CO) 16 July 1997 (1997-07-16) claims 1,2 ---	1-21
A	WO 97 27191 A (REDDY S RESEARCH FOUNDATION DR ;REDDY CHEMINOR INC (US)) 31 July 1997 (1997-07-31) page 2 -page 3 ---	1-21
A	CANCER RESEARCH, vol. 46, no. 4, 1986, pages 1735-40, XP000901165 abstract -----	1-21

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted]mation on patent family members

International Application No
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(54) Title: USE OF THIAZOLIDINEDIONES DERIVATIVES FOR PREVENTING UTERINE CONTRACTIONS IN PREMATURE LABOUR OR LACTATION

(57) Abstract

The present invention provides methods of preventing or reducing oxytocin–mediated action by using a thiazolidinedione, such as troglitazone, or thiazolidinedione–like compounds. These methods describe the employment of these compounds alone or in combination with at least one other agent, such as a tocolytic agent. This offers a novel therapeutic regimen for the treatment of oxytocin–mediated actions, for example induction of uterine contractions, prostaglandin release, and milk letdown. Accordingly, conditions such as preterm labor and labor prior to Caesarean delivery can be treated by these methods.

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DESCRIPTION

USE OF THIAZOLIDINEDIONES DERIVATIVES FOR PREVENTING UTERINE CONTRACTIONS IN PREMATURE LABOUR OR LACTATION

BACKGROUND OF THE INVENTION

This application claims the benefit of United States provisional patent application Serial No. 60/106,133 filed on October 29, 1998, which is now abandoned. The entire text of the above–referenced disclosure is specifically incorporated by reference herein without disclaimer.

I. Field of the Invention

The present invention relates generally to the field of medicine, including obstetrics. More particularly, it concerns the use of thiazolidinediones, including troglitazone, by itself and in combination with tocolytic agents to prevent or reduce oxytocin-mediated actions such as uterine contractions in premature labor and lactation.

II. Description of Related Art

Preterm birth causes at least 75% of neonatal deaths that cannot be attributed to congenital malformations. A newborn weighing less than 1500 grams is about 200 times more likely to die before becoming a year-old than an infant born with a birthweight of greater than 2500 grams. Preterm birth contributes significantly to developmental delay, visual and hearing impairment, chronic lung disease, and cerebral palsy. Low-birth-weight survivors also have a 10 times greater chance of being neurologically impaired. Even in healthy-appearing preterm infants, academic and family problems occur more frequently than they do with term infants (McCormick, 1985).

Even though preterm birth has continued to present significant problems in the field of obstetrics for many years, the rate of preterm births has remained at the same level since the middle of this century. Numerous and varied therapies are available but much controversy revolves around their efficacies in prevention of prematurity and the management of premature labor (Main, 1995). By controlling uterine contractions and allowing on time delivery, billions of dollars in care for premature infants will be saved.

Preterm labor, defined as spontaneous labor occurring prior to 37 weeks of gestation (with 39 weeks being term), accounts for approximately 1 in 10 births and is the cause of preterm delivery. Preterm delivery is associated with contractions of the uterine muscle, which are likely induced by oxytocin in the blood. Oxytocin action on the uterus is completely dependent upon the increased expression of oxytocin receptors (OTR) in the myometrium just prior to birth. The ability to inhibit the premature rise of OTR or inactivate OTR may be critical in preventing premature onset of labor and birth. The purpose of the invention is to prevent unwanted contractions by preventing an increase in OTR expression and/or preventing oxytocin from binding to OTR. Thus, the use of thiazolidinediones, such as troglitazone, or related compounds that prevent oxytocin from binding to OTR and/or inhibit an increase in OTR should prevent premature labor and labor prior to Caesarean delivery.

Known functions of oxytocin (OT) include smooth muscle contraction during birth (Fuchs *et al.*, 1982; Soloff, 1989), milk ejection during lactation (Soloff, *et al.*, 1979), and prostaglandin release (Hinko and Soloff, 1992). These actions occur as very specifically timed events because the upregulation of OTRs determines the responsiveness of cells to oxytocin. At term, myometrial OTRs rise 2-fold just before labor and fall dramatically immediately after birth. In contrast, OTRs in mammary myoepithelial cells, which contract in response to oxytocin that is reflexively released into the blood as a result of suckling, increase shortly after birth and remain elevated as long as suckling continues. From these two examples, it is clear that the rise in OTR levels dictate tissue specific OT action, and the regulation of OTRs in these two tissues is different. To date, known agents that cause an increase in OTR protein

levels include estradiol in the uterus (Fuchs, *et al.*, 1983; Larcher *et al.*, 1995), and glucocorticoids and/or cyclic AMP in rabbit amnion (Hinko and Soloff, 1993), and glucocorticoids and an unknown protein(s) in a human breast tumor cell line, Hs578T (Copland et al., 1999).

OTRs are expressed on cell surface membranes, and the binding of OT from the circulation or arising from paracrine sources sets off a cascade of intracellular events that culminate in cell contraction and/or prostaglandin synthesis. These events are mediated by G proteins tethered to the intracellular portion of OTRs (Strakova and Soloff, 1997). G_i and G_q isotypes have been shown to be coupled to OTRs, and each works through distinct and separate intracellular pathways. Activation of these G proteins results in a rapid rise in intracellular calcium, phosphorylation of mitogen-activated protein (MAP) kinase (ERK 2 and p38) (Hoare et al., 1999). Other events resulting from OT treatment include transcriptional activation of *cfos* mRNA, a protein vital for cell cycle regulation (Strakova et al., 1998). No specific competitive antagonist exists for oxytocin because oxytocin and vasopressin share a high degree of homology with one another as well as the V_{1a} vasopressin receptor and OTR (Postina et al., 1996 and references therein). Vasopressin at a 10-100 fold concentration will activate the oxytocin receptor. As well, a high affinity antagonist blocking oxytocin binding to the OTR exists but it binds equally well to the vasopressin receptor. Recently, Zingg described 5β-dihydroprogesterone, a progesterone metabolite, to noncompetively bind to the oxytocin receptor and antagonize oxytocin action (Grazzini et al., 1998). However, high concentrations of 100 μM 5β-dihydroprogesterone were needed to inhibit oxytocin induced uterine contractions (Thornton et al. 1999). A noncompetitive inhibitor (e.g. 5β-dihydroprogesterone) binds to a different site for instance on the OTR as opposed to the site that oxytocin binds to activate the OTR. A noncompetitive inhibitor alters the conformation of the molecule that it binds, thereby altering the ability of the activating ligand to bind to the same molecule. These events are only partially reversible once the noncompetitive antagonist binds. Thus, no effective specific competitive antagonist exists clinically for oxytocin and the oxytocin receptor.

By controlling uterine contractions and allowing on time delivery, billions of dollars in costs for premature infant care will be saved. For oxytocin to have biological activity, oxytocin receptors (OTR) must increase. This occurs shortly before birth as well as during breast-feeding to allow secretion of the mother's milk. Mothers who wish not to breast feed their infants could take troglitazone to inhibit oxytocin action.

Troglitazone is currently used clinically in Type 2 diabetic patients to increase insulin sensitivity and thus, increase glucose uptake into cells (thiazolidinediones do not cause hypoglycemia). This drug is taken orally with excellent absorption into the blood stream and few side effects. Troglitazone is marketed for Sankyo by Parke-Davis in the United States. No significant side effects of this drug have been demonstrated with the exception that a small percentage of patients developed idiopathic liver intolerance.

SUMMARY OF THE INVENTION

The present invention involves the interaction of thiazolidinediones, such as troglitazone, with the oxytocin receptor (OTR). Thiazolidinediones or thiazolidinedione-like compounds can be used alone, in combination with other thiazolidinediones and/or thiazolidinedione-like compounds, and/or with other compounds such as at least one tocolytic agent. The methods of the invention can be used to prevent or reduce oxytocin-mediated actions. It is contemplated that the methods described herein can be used for treating mammals, such as humans, as well as other animals.

In one embodiment of the present invention, methods are provided for preventing preterm labor in a pregnant subject by administering to the subject an amount of thiazolidinedione effective to prevent the subject from undergoing preterm labor.

In another embodiment, methods for reducing or preventing an oxytocin-mediated action in a subject comprising administering to the subject an amount of

thiazolidinedione effective to reduce the oxytocin-mediated action in the subject. The present invention can be implemented to treat any oxytocin-mediated action, including induction of labor in a pregnant subject, induction of uterine cramps, induction of milk letdown, and induction of prostaglandin release.

In other embodiments of the present invention, the thiazolidinedione compound of the treatments described above comprises troglitazone. While in further embodiments, the thiazolidinedione comprises pioglitazone, BRL49653, or a compound related to troglitazone. A compound related to troglitazone is one that is substantially similar to the chemical structure of troglitazone or can be derived from troglitazone.

The methods of the present invention have clear therapeutic and preventative applications. As such, some embodiments of the present invention include a thiazolidinedione that is dispersed in a pharmacologically acceptable form so that the thiazolidinedione can be administered to a subject. Administration of the compound could be accomplished locally, parenterally, intravenously, or intravaginally. It is contemplated that intravaginal administration through the use of, for example, a suppository or cream formulation, provides therapeutic benefits for the treatment of uterine contractions.

While the methods of the present invention employs thiazolidinedione alone, it is further contemplated that the methods can be implemented using thiazolidinedione in combination with at least one other thiazolidinedione, a thiazolidinedione-like compound, or a tocolytic agent. Tocolytic agents have been used to relax the uterus, and in some embodiments of the present invention, the tocolytic agent comprises at least one beta-mimetic, magnesium sulfate, at least one prostaglandin inhibitor, or at least one calcium-blocking agent. In still further embodiments of the described invention, the prostaglandin inhibitor is indomethacin, whereas the calcium-blocking agent is nifedipine.

In the combination treatments of the present invention using both a thiazolidinedione and a tocolytic agent, in one embodiment of the invention, the

compounds are administered to a subject at the same time, but in other embodiments, the thiazolidinedione is administered before the other tocolytic agent, and vice versa.

The present invention is also directed at methods of screening for antagonists and agonists of oxytocin since troglitazone was shown herein to bind the oxytocin receptor. In some embodiments, screening for an oxytocin agonist is done by administering a troglitazone-like compound to an oxytocin receptor and determining whether the compound binds the receptor. Measurable binding identifies the troglitazone-like compound as an agonist. In other embodiments, screening for an oxytocin antagonist is accomplished by at least (a) administering a thiazolidinedione to an oxytocin receptor; (b) administering a composition comprising a candidate oxytocin antagonist; and (c) determining whether the thiazolidinedione binds to the receptor.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. 1. Binding of thiazolidinediones to oxytocin receptors. Percent inhibition of ^{125}I-OTA binding in human myometrial cells.

FIG. 2. Prostaglandin E_2 levels in primary human myometrial cells. Cells were treated with oxytocin with or without troglitazone for 20 hours. Media was removed and frozen until assayed for PGE2 levels.

FIG. 3A-H. Change in intracellular cytoplasmic calcium levels in human term myometrial cells in culture. Each line represents the change in calcium for a

single cell. Cells were either pretreated with troglitazone for one minute and then treated with oxytocin, bombesin (BBS), or bradykinin. These three agents cause an increase in intracellular calcium and cause uterine contraction under the correct circumstance. Cells were grown in 10% FBS, DMEM, penicillin/streptomycin resulting in elevated OTR levels. Cells were then put into KRH buffer containing Fura-2. The cells take up Fura-2 allowing for detection in changes of cytoplasmic levels of calcium. Cells were then treated as described for each figure. In figure 3A, cells were treated with 10 nM oxytocin demonstrating an increase in cytoplasmic calcium levels. In figure 3B, cells were pretreated with 0.001 microgram/ml troglitazone (2.2 nM) 1 minute prior to 10 nM oxytocin treatment. There is no effect of troglitazone at this concentration upon oxytocin-stimulated calcium transients. In FIG. 3C, a 0.01 microgram/ml (22.6 nM) troglitazone partially blocks oxytocin-induced calcium transients. In FIG. 3D, 0.1 microgram/ml (226 nM) troglitazone completely blocks 10 nM oxytocin-induced calcium transients but does not block 100 nM bombesin-induced calcium transients. This demonstrates specificity of troglitazone for oxytocin mediated action (also shown in FIG. 3G). In figure 3E, 100 nM oxytocin and 100 nM bradykinin induce a calcium transient in human myometrial cells. In FIG. 3F a 226 nM troglitazone does not block calcium transients induced by oxytocin and bradykinin whereas in FIG. 3G, 1 microgram/ml (2.2 micromolar) troglitazone specifically blocks oxytocin- but not bradykinin-induced calcium transients. In FIG. 3H, 100 nM and 1 micromolar troglitazone demonstrate a dose-dependent inhibition of the rise in oxytocin-induced calcium levels.

FIG. 4A and FIG. 4B. Measurement of uterine contractions from term human myometrial tissue from C-sections. Rhythmic contractions occur at approximately 3-4 minute intervals as shown in FIG. 4A and 4B. Upon stimulation with 10 nM oxytocin, myometrium tissue has multiple contractions as shown in figure 4A. After approximately 5 minutes (Distance between each verticle axis line represents a minute.), uterine contractions cease in response to 10 nM oxytocin. Pretreatment of myometrium with 10 micrograms/ml (22.6 micromolar) troglitazone for one minute blocks 10 nM oxytocin stimulated contractions (figure 4B). After 5 minutes, 100 nM oxytocin was introduced. Contractions occurred in the presence of

100 nM oxytocin demonstrating that the process is reversible. This indicates that competition for binding to the oxytocin receptor occurs.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

The present invention is based on the observation that thiazolidinediones, such as troglitazone, can inhibit the binding of oxytocin to the oxytocin receptor (OTR). Oxytocin binding triggers various oxytocin-mediated activities such as the onset of uterine contractions, and concomitantly the onset of labor, prostaglandin release, and milk letdown. Oxytocin causes prostaglandin release from the ovary and uterus. Prostaglandin E_2 and F_α may play a role in luteolysis and luteal phase defect. Thiazolidinediones, such as troglitazone, and thiazolidinedione-like compounds thus provide a therapeutic and prophylactic approach to inhibit any unwanted action of oxytocin or similar compounds that activates OTR, *e.g.*, vasopressin. Exemplary thiazolidinediones are seen in U.S. Patent 5,478,852 and U.S. 5,814,647, which are incorporated by reference herein.

The value of these treatments is large since approximately 10% of pregnant women deliver preterm (Norton *et al.*, 1993; Main, 1995). To date, no studies convincingly demonstrate improved survival or any index of longterm neonatal outcome with the use of existing tocolytic (uterine relaxing) therapy. Many potential damages of tocolytic therapy to mother and neonate are well documented (Norton *et al.*, 1993; Moise *et al.*, 1998; Wilkins et al., 1988). Potential complications of beta-adrenergic agents include hyperglycemia, hypokalemia, hypotension, pulmonary edema, cardiac insufficiency, dysrhythmias, myocardial ischemia, and maternal death. Potential complications of magnesium sulfate include pulmonary edema, respiratory depression, cardiac arrest, maternal tetany, profound muscular paralysis, and profound hypotension. All but pulmonary edema are rare complications of this treatment. Potential complications of nifedipine treatment include transient hypotension. Thus, it is clear that additional methods for the treatment of preterm deliveries are needed.

I. Oxytocin-Mediated Actions

It is the inventors' discovery that thiazolidinediones, such as troglitazone, can bind to the oxytocin receptor (OTR), and consequently, the present invention comprises inhibiting or reducing oxytocin-mediated actions.

Oxytocin is a short-lived, fast acting hormone, made by the hypothalamus of the brain, along with its close relative vasopressin (anti-diuretic hormone), stored in the posterior pituitary, and released into the blood as needed. It stimulates certain smooth muscle coats, constricts certain blood vessels and facilitates the sensitivity of some tissues to other hormones and nerves. The main tissues affected are the uterus, including endometrium and myometrium, vagina, breasts (both sexes), erectile tissue (both sexes), seminal vesicles, and with special-case effects on uterine muscle contractions in both birth and orgasm, the vascular constriction that lessens placental separation bleeding, and the let-down reflex that nursing mothers have when babies cry.

Oxytocin is produced in two discrete groups of neurons in the brain of all mammals. One group of oxytocin-producing neurons projects to the posterior pituitary, which is an endocrine gland located at the base of the brain. From the pituitary, oxytocin is released into the bloodstream, whereby it exerts the well-known peripheral effects like uterine contraction and milk let-down. The other group of oxytocin-containing neurons projects directly to specific brain areas that are known to mediate maternal behaviors. By acting locally as a chemical messenger in these brain areas, oxytocin acts as a regulator or controller of maternal behaviors.

Agents known to stimulate the release of oxytocin from the posterior pituitary include sensory stimuli arising from the cervix, vagina, and breast. Secretion of oxytocin is also stimulated by increases in the osmality of plasma. Secretion of oxytocin is suppressed by ethanol and ovarian relaxin. The present invention contemplates the use of agents that stimulate the release of endogenous oxytocin, as described above, as well as antagonists of agents that normally suppress the release of endogenous oxytocin.

Oxytocin is currently indicated for stimulation of uterine contraction to induce labor and for the control of postpartum hemorrhage following delivery of the placenta. It is also indicated for stimulation of lactation for breast-feeding. Oxytocin is currently prepared synthetically and sold under various trade names including Pitocin (Parke-Davis, Morris Plains, NJ) and Syntocinon. It can be administered intravenously, intramuscularly, and by nasal absorption. Activity of oxytocin is expressed in terms of USP units, as defined in a bioassay of uterine-stimulating potency of posterior pituitary extracts. One USP unit is the equivalent of approximately 2 μg of pure peptide.

Oxytocin receptors (OTRs) are expressed on the cell surface membrane. The first three extracellular domains of OTR are crucial for high-affinity oxytocin binding and for selection of agonists (Postina *et al.*, 1996). Oxytocin from the circulation or arising from paracrine derived sources interact with cell surface OTRs to set off a cascade of intracellular events. These events are mediated by G proteins tethered to the intracellular portion of OTRs (Strakova and Soloff, 1997). Subsequent activation of these G proteins result in a rapid rise in intracellular calcium and phosphorylation of MAP kinases (ERK 2). Other events resulting from oxytocin treatment include transcriptional activation of *cfos* mRNA, a protein vital for cell cycle progression (Strakova et al., 1998).

Oxytocin and oxytocin related compounds, acting through oxytocin receptors, are currently in clinical use for induction of uterine contractions and facilitation of delivery of a baby and placenta at the time of birth. This action is dependent upon the timely increase of OTRs (oxytocin receptors) on the target cell surface. Without an upregulation of OTRs, oxytocin has no action on the parturient uterus, thus limiting adverse side effects. During the latter stages of pregnancy, the number of OTRs increases, which ultimately causes the smooth muscle of the uterus to contract and lead to labor. The interaction between oxytocin and the OTR is also involved with uterine cramping generally, the promotion of milk glands to release milk (milk letdown), and prostaglandin release.

Examples of oxytocin agonists that would be preferred in the present invention include 4-threonine-1-hydroxy-deaminooxytocin, 9-Deamidooxytocin, an analog of oxytocin containing a glycine residue in place of the glycinamide residue (Ferrier and Du Vigneaud, 1966); 7-D-proline-oxytocin and its deamino analog (Ferraro and Du Vigneaud, 1966); (2,4-Diisoleucine)-oxytocin, an analog of oxytocin with natriuretic and diuretic activities (Hruby *et al.*, 1970); deamino oxytocin analog (Urry *et al.*, 1970); a long-acting oxytocin (OT) analog 1-desamino-1-monocarba-E12-Tyr(OMe)]-OT(dCOMOT) (Veznik *et al.*, 1979; Cort *et al.*, 1982 and 1979); carbetocin, a long-acting oxytocin analog (Hunter *et al.*, 1992); oxytocin agonist [Thr4-Gly7]-oxytocin (TG-OT) (Chadio and Antoni, 1993); oxytocin agonist as described by Olson *et al.*, (1991); oxypressin, an equipotent analog of oxytocin and vasopressin (Gazis *et al.*, 1987); and Deamino-6-carba-oxytoxin (dC60), a potent oxytocin analog considered to be resistant to some of the physiologically significant enzymic systems (Krejci *et al.*, 1981). As well, nonpeptide oxytocin antagonists have been recently been described which include L-371,257, related series of compounds containing an ortho-trigluoroethoxyphenylacetyl core (e.g. L374,943) (Williams et al., 1999).

U.S. Patent 5,846,766 relates to a receptor for a posterior pituitary hormone, oxytocin; a DNA sequence encoding for the receptor; a recombinant DNA molecule containing the DNA sequence and a transformant comprising the recombinant DNA molecule. The present invention further relates to methods of detection and diagnosis and a kit to aid in same which comprise either oxytocin, its receptor or antibodies to the receptor.

A. Preterm Labor/Caesarean Delivery

The present invention includes methods of preventing or reducing the risk of preterm delivery through the administration of thiazolidinediones such as troglitazone to a pregnant subject. Major changes in the function and structure of the uterus occur with respect to pregnancy. Until labor occurs, the uterus is mainly quiescent. At that point, heightened contractile activity involves raised levels of (i) oxytocin receptors; (ii) calcium channels; (iii) gap junctions, and (iv) endothelin receptors.

Preterm labor is generally characterized by regular uterine contractions accompanied by progressive cervical dilation and/or effacement prior to week 37 of gestation in humans. In most cases, the cause of preterm labor is not known, though some predisposing factors have been identified. Various circumstances such as multiple gestations and a history of second-trimester simultaneous abortion have been associated with its occurrence, in addition to some maternal activities.

Some methods exist for determining whether a patient is at risk for preterm delivery. One method involves testing for the presence of fetal fibronectin in the pregnant mother's cervical or vaginal secretions, which is an indicator of the possibility of preterm delivery (Lockwood *et al.*, 1991). Alternatively, levels of salivary estriol has been used to distinguish true and false labor (U.S. Patent No. 5,480,776) and to determine the effectiveness of tocolytic therapies for the postponement of labor (U.S. Patent No. 5,370,135).

Some preventative therapies have been studied, although the efficacy of none of the approaches has been unequivocally established. For example, oral tocolytic therapy has been prescribed to prolong gestation, as has reduced activity for the mother. Thus, the present invention provides a much needed alternative to existing treatments in the prevention of preterm labor. Management of preterm labor has also been a subject of study where tocolysis treatments have been utilized with various degrees of effectiveness. No study has proven the long-term neonatal benefits of this therapy. Likewise, the present invention has utility with respect to management of preterm labor, by a regimen comprising at least one thiazolidinedione alone or in combination with other tocolytic agents.

As the present invention can inhibit or reduce uterine contractions through the administration of thiazolidinedione, such as troglitazone, or thiazolidinedione-like compounds, the methods of the present invention can be used to inhibit uterine contractions prior to Caesarean delivery (C-section), which refers to a surgical procedure in which an incision is made through the abdominal and uterine walls for delivery of a fetus. Furthermore, the methods of the invention can be used to treat dysmenorrhea, which describes painful menstruation.

B. Other Oxytocin-mediated Actions

Another observation of the present invention relates to the ability of thiazolidinediones to prevent the release of prostaglandins by oxytocin. Prostaglandins comprise modified fatty acids that have a number of activities. Prostaglandins are cyclic, unsaturated fatty acids that are usually derived from arachidonic acid, a 20-carbon, straight-chain, polyunsaturated fatty acid precursor. Prostaglandins can act as potent vasodilators to relax muscles in the walls of blood vessels. In women, prostaglandins are involved in the control of gonadotropin releasing hormone (GnRH) over luteinizing hormone (LH) secretion, control of ovulation, and inducement of uterine contractility. Recent studies have indicated that inhibiting prostaglandin release can be an effective treatment for dysmenorrhea, while administration of prostaglandin results in inducement of labor or therapeutic abortions. Prostaglandins have also been implicated in the progression of cancer. As well, many tissues in which prostaglandins play a role in cancer progression such as colon and breast may also express oxytocin receptors. Recently, oxytocin receptors have been described in human breast tumors. Thus, an oxytocin antagonist may slow tumor progression by inhibiting prostaglandin synthesis.

To date, oxytocin is known to signal through calcium and MAP kinase pathways. New, not yet to be described pathways may be discovered that play critical roles in cell differentiation, cell proliferation, and apoptosis. A pure oxytocin antagonist could elucidate the role of oxytocin in these pathways.

C. Tocolytic Agents

In some embodiments of the present invention, a therapy to inhibit or reduce oxytocin-mediated action comprises administering a thiazolidinedione such as troglitazone and another tocolytic agent. A number of tocolytic (uterine relaxing) agents are in use to prevent uterine contractions. These include beta-mimetics, such as β-adrenoreceptor stimulants (for example, salbutamol, terbutaline, isoxsuprine, ritodrine, and fenoterol), magnesium sulfate, prostaglandin inhibitors (such as, indomethacin, aspirin, and naproxen), and calcium-blocking agents like nifedipine and

nicardipine. Additionally, a candidate for tocolysis is the calcitonin gene-related peptide (CGRP), which is a powerful vasodilator that generally relaxes smooth muscle tissue and has been recently reported as inducing dose-dependent relaxation of spontaneously contracting myometrium in pregnant women (Dong *et al.*, 1999; Yallampi *et al.*, 1999, both incorporated herein by reference). It is contemplated that the thiazolidinedione and the other tocolytic agent may be administered simultaneously or at different times to inhibit or reduce uterine contractions or any other oxytocin-mediated action.

Tocolysis treatment usually employs the least amount of the tocolytic agent to effect reduction in the frequency of uterine contractions and stop cervical alterations. Tocolytic agents such as ritodrine and magnesium sulfate are generally given intravenously. Terbutaline, a beta-mimetic, is administered intravenously or subcutaneously. If intravenous treatment is successful, this is typically followed up with oral administration of ritodrine or terbutaline. Oral therapy can be used to maintain the effect until 35 to 37 weeks of gestation.

General contraindications to tocolysis for preterm labor include the following: acute fetal distress, chorioamnionitis, eclampsia or severe preeclampsia, fetal demise, fetal maturity, and maternal hemodynamic instability. Moreover, the following complications have been observed: for beta-adrenergic agents (beta-mimetics), hyperglycemia, hypokalemia, hypotension, pulmonary edema, cardiac insufficiency, arrhythmias, myocardial ischemia, and maternal death; for magnesium sulfate, pulmonary edema, respiratory depression, cardiac arrest, maternal tetany, profound muscular paralysis, and profound hypotension; for indomethacin, hepatitis, renal failure, and gastrointestinal bleeding; and for nifedipine, transient hypotension.

II. Troglitazone and Other Thiazolidinediones

The methods of the present invention are directed at methods of inhibiting or reducing oxytocin-mediated action and generally treating a subject through the use of troglitazone, other thiazolidinediones, or thiazolidinedione-like compounds, some of which are described in U.S. Patent Number 5,968,960, which is hereby incorporated by reference. Methods of making thiazolidinediones and thiazolidinedione-like

compounds are described, for example, in U.S. Patent No. 5,223,522 issued Jun. 29, 1993; U.S. Pat. No. 5,132,317 issued Jul. 12, 1992; U.S. Pat. No. 5,120,754 issued Jun. 9, 1992; U.S. Pat. No. 5,061,717 issued Oct. 29, 1991; U.S. Pat. No. 4,897,405 issued Jan. 30, 1990; U.S. Pat. No. 4,873,255 issued Oct. 10, 1989; U.S. Pat. No. 4,687,777 issued Aug. 18, 1987; U.S. Pat. No. 4,572,912 issued Feb. 25, 1986; U.S. Pat. No. 4,287,200 issued Sept. 1, 1981; U.S. Pat. No. 5,002,953, issued Mar. 26, 1991; U.S. Pat. Nos. 5,972,944; 5,965,589; 5,910,592; 5,811,439; 5,506,245; 4,340,605; 4,438,141; 4,444,779; 4,461, 902; 4,703,052; 4,725,610; 4,897,393; 4,918,091; 4,948,900; 5,194,443; 5,232,925; and 5,260,445; WO 91/07107; WO 92/02520; WO 94/01433; WO 89/08651; and JP Kokai 69383/92, which are herein incorporated by reference.

Thiazolidinedione-like compounds include compounds with structural similarity to thiazolidinedione. Such a thiazolidinedione-like compound may comprise, or be composed entirely of, at least one derivative or mimic of at least one moiety that may be present in a thiazolidinedione. As used herein a "derivative" or "thiazolidinedione-like compound" refers to a chemically modified or altered form of a thiazolidinedione molecule, while the terms "mimic" or "analog" refers to a molecule that may or may not structurally resemble a thiazolidinedione molecule, but functions similarly to it. As used herein, a "moiety" generally refers to a smaller chemical or molecular component of a larger chemical or molecular structure, and is encompassed by the term "molecule."

Troglitazone (±-5-[[4-[(3,4-dihydro-6-hydroxy-2,5, 7, 8-tetramethyl-2H-1-benzopyran-2-yl)methoxy]phenyl]methyl]-2,4-thiazolidinedione) has been used in the management of Type II diabetes as an antihyperglycemic agent. Insulin sensitivity is enhanced by it in muscle and adipose tissue, and troglitazone impedes hepatic gluconeogenesis. It has been used alone or in combination with a sulfonylurea to control adult-onset diabetes. It has a molecular weight of approximately 441 daltons and its formula is $C_{24}H_{27}NO_8S$. Polymorphic forms of troglitazone have also been described, such as in United States Patent No. 5,700,820, which is herein incorporated by reference. BRL49563 (rosiglitazone BRL49653 or avandia, Smith-Kline recently introduced on market) and pioglitazone are second generation thiazolidinediones used

in the treatment of type 2 diabetes. They were designed based on the structure of troglitazone and differ in that these two compounds do not contain the vitamin E moiety that is contained in troglitazone. BRL49653 and have a 100-and 6-fold higher affinity for the transcriptional factor PPARγ compared to that of troglitazone. Thus, in type 2 diabetes, 2-4 mg/day of BRL49563 (avandia, rosiglitazone) are used in patients as compared to 200-400 mg/day of troglitazone to lower blood glucose levels. It is important to note that thiazolidinediones do not cause hypoglycemia. These agents act to sensitize insulin's ability to cause blood glucose to be taken up into target cells *e.g.* adipocytes and muscle.

III. Methods for Assaying Troglitazone or Other Thiazolidinedione Activity

Because this invention is based on the observation that thiazolidinediones such as troglitazone both inhibit oxytocin from binding its receptor and bind to the receptor, binding assays present an embodiment for assaying thiazolidinedione activity. These binding assays are well known to those of skill in the art, as represented by Hoare *et al.*, 1999 and Postino *et al.*, 1996, which are herein incorporated by reference. Such assays can be used to evaluate the efficacy of thiazolidinedione therapy; alternatively, these assays can be used with the screening methods of the present invention to identify and characterize agonists and antagonists of oxytocin. An "agonist" is used herein to refer to a compound or substance that can bind to a receptor and activate a signalling pathway. With respect to the present invention, an agonist would be capable of binding the oxytocin receptor and mimic the function of oxytocin. Because thiazolidinediones bind OTRs, it is contemplated that the structure of thiazolidinediones can serve the basis for this interaction. Other thiazolidinedione-like substances may serve as agonists, and any oxytocin agonist activities can be evaluated in an OTR binding assay.

An "antagonist" of the present invention is a substance or compound that competes with another substance, such as a thiazolidinedione, for binding to a receptor, for example the OTR. Antagonists can be screened according to the present invention by assaying for competition between the candidate antagonist and a thiazolidinedione for binding to the OTR. Competition is qualified as any detectable

displacement of thiazolidinedione by the candidate for OTR binding. In rats and possibly humans, progesterone, for example, has been observed to possess oxytocin antagonist activity using binding assays (Thornton *et al.*, 1999).

IV. Methods for Blocking Oxytocin-Mediated Actions

The present invention is directed at methods of inhibiting or reducing oxytocin-mediated action and generally treating a subject through the administration of troglitazone, other thiazolidinediones, or thiazolidinedione-like compounds. Consequently, formulations and routes of administration for the compounds are described below.

A. Formulations and Routes for Administration to Patients

Where clinical applications are contemplated, it will be necessary to prepare pharmaceutical compositions in a form appropriate for the intended application. Generally, this will entail preparing compositions that are essentially free of pyrogens, as well as other impurities that could be harmful to humans or animals.

One will generally desire to employ appropriate salts and buffers to render delivery vectors stable and allow for uptake by target cells. Buffers also will be employed when recombinant cells are introduced into a patient. Aqueous compositions of the present invention comprise an effective amount of the thiazolidinedione to cells, dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium. Such compositions also are referred to as inocula. The phrases "pharmaceutically acceptable" or "pharmacologically acceptable" refer to molecular entities and compositions that do not produce adverse, allergic, or other untoward reactions when administered to an animal or a human. As used herein, "pharmaceutically acceptable carrier" or "pharmacologically acceptable carrier" include any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents and the like. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the vectors or cells of

the present invention, its use in therapeutic compositions is contemplated. Supplementary active ingredients also can be incorporated into the compositions.

The inventors propose that local, regional delivery of thiazolidinedione, such as troglitazone, to a patient who may experience or is experiencing an oxytocin-mediated action, such as uterine contractions in labor or lactation, will be a very efficient method for delivering a therapeutically effective composition to counteract the oxytocin action. Similarly, tocolytic agents may be directed to a particular, affected region of the subject's body. Regional administration includes administration via intra-arterial, intracavity, intravaginal, intravesical, intrathecal, intrapleural, and intraperitoneal routes.

The active compositions of the present invention may include classic pharmaceutical preparations. Administration of these compositions according to the present invention will be via any common route so long as the target tissue is available via that route. This includes oral, nasal, buccal, rectal, vaginal or topical. Alternatively, administration may be by orthotopic, intradermal, subcutaneous, intramuscular, intraperitoneal or intravenous injection. Such compositions would normally be administered as pharmaceutically acceptable compositions, described *supra*. The drugs and agents also may be administered parenterally or intraperitoneally. The term "parenteral" is generally used to refer to drugs given intravenously, intramuscularly, or subcutaneously.

The biological material should be extensively dialyzed to remove undesired small molecular weight molecules and/or lyophilized for more ready formulation into a desired vehicle, where appropriate. The active compounds will then generally be formulated for parenteral administration, *e.g.*, formulated for injection via the intravenous, intramuscular, sub-cutaneous, intralesional, or even intraperitoneal routes. The preparation of an aqueous composition that contains a thiazolidinedione or thiazolidinedione-like composition as an active component or ingredient will be known to those of skill in the art in light of the present disclosure. Typically, such compositions can be prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for using to prepare solutions or suspensions upon the addition of

a liquid prior to injection can also be prepared; and the preparations can also be emulsified.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

The therapeutic compositions of the present invention are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per milliliter of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, *etc.* Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH, exact concentration of the various components, and the pharmaceutical composition are adjusted according to well known parameters. Suitable excipients for formulation with a thiazolidinedione, such as troglitazone, include croscarmellose sodium, hydroxypropyl methylcellulose, iron oxides (synthetic), magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, polysorbate 80, povidone, silicon dioxide, titanium dioxide, and water (purified).

Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions also can be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

A thiazolidinedione or thiazolidinedione–like composition can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally,

dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The preparation of more, or highly, concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents to a small area.

Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The thiazolidinedione or thiazolidinedione-compound may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. In preferred embodiments, the active oxytocin or oxytocin analog are formulated within a therapeutic mixture to comprise about 0.001 to about 1 milligram. Multiple doses can also be administered.

The therapeutic compositions of the present invention are advantageously administered in the form of injectable compositions either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. These preparations also may be emulsified. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. For instance, the composition may contain 10 mg, 25 mg, 50 mg or up to about 100 mg of human serum albumin per milliliter of phosphate buffered saline. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, *etc.* Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobial agents, anti-oxidants, chelating agents and inert gases. The pH, exact concentration of the various components, and the pharmaceutical composition are adjusted according to well known parameters. Suitable excipients for formulation with a thiazolidinedione, such as troglitazone, include croscarmellose sodium, hydroxypropyl methylcellulose, iron oxides (synthetic), magnesium stearate, microcrystalline cellulose, polyethylene glycol 400, polysorbate 80, povidone, silicon dioxide, titanium dioxide, and water (purified).

In addition to the compounds formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, *e.g.*, tablets or other solids for oral administration; liposomal formulations; time release capsules; and any other form currently used, including creams.

One may also use nasal solutions or sprays, aerosols or inhalants in the present invention. Nasal solutions are usually aqueous solutions designed to be administered to the nasal passages in drops or sprays. Nasal solutions are prepared so that they are similar in many respects to nasal secretions, so that normal ciliary action is maintained. Thus, the aqueous nasal solutions usually are isotonic and slightly buffered to maintain a pH of 5.5 to 6.5.

In addition, antimicrobial preservatives, similar to those used in ophthalmic preparations, and appropriate drug stabilizers, if required, may be included in the formulation. Various commercial nasal preparations are known and include, for example, antibiotics and antihistamines and are used for asthma prophylaxis.

Additional formulations that are suitable for other modes of administration include vaginal suppositories and pessaries. A rectal pessary or suppository may also be used.

Suppositories are solid dosage forms of various weights and shapes, usually medicated, for insertion into the rectum, vagina or the urethra. After insertion, suppositories soften, melt or dissolve in the cavity fluids.

In general, for suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%.

Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders. Additional formulations are suitable for oral administration. Oral formulations include such typical excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions,

tablets, pills, capsules, sustained release formulations or powders. When the route is topical, the form may be a cream, ointment, salve or spray.

In certain embodiments, oral pharmaceutical compositions will comprise an inert diluent or assimilable edible carrier, or they may be enclosed in hard or soft shell gelatin capsule, or they may be compressed into tablets, or they may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tables, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 75% of the weight of the unit, or preferably between 25-60%. The amount of active compounds in such therapeutically useful compositions is such that a suitable dosage will be obtained.

The tablets, troches, pills, capsules and the like may also contain the following: a binder, as gum tragacanth, acacia, cornstarch, or gelatin; excipients, such as dicalcium phosphate; a disintegrating agent, such as corn starch, potato starch, alginic acid and the like; a lubricant, such as magnesium stearate; and a sweetening agent, such as sucrose, lactose or saccharin may be added or a flavoring agent, such as peppermint, oil of wintergreen, or cherry flavoring. When the dosage unit form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup of elixir may contain the active compounds sucrose as a sweetening agent methyl and propylparabens as preservatives, a dye and flavoring, such as cherry or orange flavor.

B. Therapeutically Effective Amounts of Troglitazone and Other Thiazolidinediones

An effective amount of the therapeutic agent is determined based on the intended goal, for example (i) inhibition of uterine contractions or (ii) inhibition of milk letdown. The term "unit dose" refers to physically discrete units suitable for use in a subject, each unit containing a predetermined-quantity of the therapeutic composition calculated to produce the desired responses, discussed above, in association with its administration, *i.e.*, the appropriate route and treatment regimen. The quantity to be administered, both according to number of treatments and unit dose, depends on the subject to be treated, the state of the subject and the protection desired. Precise amounts of the therapeutic composition also depend on the judgment of the practitioner and are peculiar to each individual.

A therapeutically effective amount of a thiazolidinedione, such as troglitazone, that may be combined with a second tocolytic agent as treatment varies depending upon the host treated and the particular mode of administration. In one embodiment of the invention the dose range of thiazolidinedione such as troglitazone used will be about 0.5mg/kg body weight to about 500mg/kg body weight. The term "body weight" is applicable when an animal is being treated. When isolated cells are being treated, "body weight" as used herein should read to mean "total cell weight". The term "total weight may be used to apply to both isolated cell and animal treatment. All concentrations and treatment levels are expressed as "body weight" or simply "kg" in this application are also considered to cover the analogous "total cell weight" and "total weight" concentrations. However, those of skill will recognize the utility of a variety of dosage range, for example, 1mg/kg body weight to 450mg/kg body weight, 2mg/kg body weight to 400mg/kg body weight, 3mg/kg body weight to 350mg/kg body weighty, 4mg/kg body weight to 300mg/kg body weight, 5mg/kg body weight to 250mg/kg body weighty, 6mg/kg body weight to 200mg/kg body weight, 7mg/kg body weight to 150mg/kg body weighty, 8mg/kg body weight to 100mg/kg body weight, or 9mg/kg body weight to 50mg/kg body weight. Further, those of skill will recognize that a variety of different dosage levels will be of use, for example, 1mg/kg, 2mg/kg, 3mg/kg, 4mg/kg, 5mg/kg, 7.5mg/kg, 10, mg/kg, 12.5mg/kg, 15mg/kg,

17.5mg/kg, 20mg/kg, 25mg/kg, 30mg/kg, 35mg/kg, 40mg/kg, 45 mg/kg, 50mg/kg, 60mg/kg, 70mg/kg, 80mg/kg, 90mg/kg, 100mg/kg, 120mg/kg, 140mg/kg, 150mg/kg, 160mg/kg, 180mg/kg, 200mg/kg, 225 mg/kg, 250mg/kg, 275mg/kg, 300mg/kg, 325mg/kg, 350mg/kg, 375mg/kg, 400mg/kg, 450mg/kg, 500mg/kg, 550mg/kg, 600mg/kg, 700mg/kg, 750mg/kg, 800mg/kg, 900mg/kg, 1000mg/kg, 1250mg/kg, 1500mg/kg, 1750mg/kg, 2000mg/kg, 2500mg/kg, and/or 3000mg/kg. Of course, all of these dosages are exemplary, and any dosage in-between these points is also expected to be of use in the invention. Any of the above dosage ranges or dosage levels may be employed for troglitazone or other thiazolidinediones alone or for such a compound in combination with a tocolytic drug.

"Therapeutically effective amounts" or ""amount of [a compound] effective" refer to those amounts effective to produce beneficial results, such as inhibition or reduction of oxytocin-mediated actions. Such amounts may be initially determined by reviewing the published literature, by conducting *in vitro* tests or by conducting metabolic studies in healthy experimental animals. Before use in a clinical setting, it may be beneficial to conduct confirmatory studies in an animal model, preferably a widely accepted animal model of the particular disease to be treated. Preferred animal models for use in certain embodiments are rodent models, which are preferred because they are economical to use and, particularly, because the results gained are widely accepted as predictive of clinical value.

As is well known in the art, a specific dose level of active compounds such as troglitazone and thiazolidinediones compounds for any particular patient depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, rate of excretion, drug combination, and the severity of the particular disease undergoing therapy. The person responsible for administration will determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, general safety and purity standards as required by FDA Office of Biologics standards.

In some embodiments, the troglitazone or thiazolidinediones compound will be administered in combination with a second agent. So long as a dose of second agent that does not exceed previously quoted toxicity levels is not required, the effective amounts of the second agents may simply be defined as those amounts effective to inhibit or reduce oxytocin-mediated actions, when administered to an animal in combination with the thiazolidinedione. This is easily determined by monitoring the animal or patient and measuring those physical and biochemical parameters of health and disease that are indicative of the success of a given treatment. Such methods are routine in animal testing and clinical practice.

C. Combination Therapies

A major purpose of the invention is to reduce oxytocin-mediated action. Administration of thiazolidinedione or thiazolidinedione-like compounds alone or in combination with other agents is contemplated. Other agents that can be combined with a thiazolidinedione or thiazolidinedione-like compound include other tocolytic agents, such as beta mimetics, magnesium sulfate, prostaglandin inhibitors, and calcium-blocking agents that relax the uterus will reduce or prevent oxytocin-mediated actions that affect uterine contractions, prostaglandin release, and milk letdown.

Various combinations may be employed; thiazolidinedione or a thiazolidinedione-like compound is "A" and the other agent is "B":

A/B/A B/A/B B/B/A A/A/B A/B/B B/A/A A/B/B/B B/A/B/B

B/B/B/A B/B/A/B A/A/B/B A/B/A/B A/B/B/A B/B/A/A

B/A/B/A B/A/A/B A/A/A/B B/A/A/A A/B/A/A A/A/B/A

V. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by

the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like similar result without departing from the spirit and scope of the invention.

EXAMPLE 1

Materials and Methods

Chemicals: Chemicals were obtained from the following sources: OT and OT antagonist (OTA) = [d(CH_2)$_5$, Tyr(Me)^2, Thr^4, Tyr-NH_2^{9}] OVT, Peninsula Laboratories (Belmont, CA); ascorbic acid, dexamethasone, and β-glycerophosphate, Sigma.

Cell Culture Conditions: The proper protocols were followed in obtaining human term myometrium tissue from mother's delivering via C-section. Human term pregnant myometrial cells were isolated by collagenase dispersion and were grown in media consisting of Delbecco's modified eagles media (DMEM) supplemented with 10% fetal bovine serum (FBS), and 2% penicillin/streptomycin in a humidified tissue culture incubator at 37°C under an atmosphere of 5% CO_2 and 95% air.

Determination of OTR ligand binding: OTA was monoiodinated as previously described (Hinko and Soloff, 1992). The specific activity of the iodinated peptide was 2000 Ci/mmol at the time of preparation. Whole cell assays for specific OTR binding activity were performed as described previously, using increasing concentrations of [^{125}I]OTA (Copland *et al.*, 1999). The concentration of cellular DNA was determined in parallel, using the Hoechst dye H 33258 and a Hoefer DyNA Quant fluorometer according to the manufacturer's instructions.

For competition studies, the appropriate concentration of compound e.g. troglitazone, BRL49653, pioglitazone, estradiol, and vitamin E succinate were incubated in the presence of radiolabeled ^{125}I-OTA. The specific binding was determined by subtracting total cpm's from nonspecific binding. Specific binding was expressed as a percent of total binding. All points were done in triplicate.

Intracellular calcium levels: Real-time recording of $[Ca^{2+}]i$ was performed in single cells using methods and design previously described (Copland *et al.*, 1999). The sensitivity of the assay was 2.5 pg/ml with an intraassay coefficient of variation of 6.3% and the interassay coefficient of variation of 6.9%.

Prostaglandin E2 (PGE2) levels: PGE2 levels in media were measured using a PGE2 enzyme immunoassay (EIA) system from Amersham Life Sciences (Arlington Heights, IL) as previously described (Copland, et al., 1999). The sensitivity of the assay was 2.5 pg/ml with an intraassay coefficient of variation of 6.3% and the interassay coefficient of variation of 6.9%.

Statistics: One way analysis of variance followed by Newman-Keuls test were used to determine statistical differences between the means of the different treatment groups (Statview 512 software, BrainPowers, Inc., Calabasas, CA). Differences were considered to be significant at $P<0.05$ level.

EXAMPLE 2

Interaction between Troglitazone and an Oxytocin Receptor

In FIG. 1, troglitazone is shown to bind directly to OTR. When added to the binding assay, troglitazone competes directly with ^{125}I-OTA to decrease levels of detectable binding as the dose of troglitazone is increased. The 50% inhibitory concentration (IC_{50}) for troglitazone is 15.7 nM as compared to oxytocin which has an IC_{50} = 0.58 nM. The IC_{50} for BRL49653 and pioglitazone are approximately 10.6 μM. The IC_{50} for 5β-dihydroprogesterone is approximately 100 μM. Vitamin E succinate and 17β-estradiol demonstrate no affinity for OTR binding.

EXAMPLE 3

Interference of Oxytocin-mediated Activities by Troglitazone

Examining prostaglandin E_2 (PGE_2) synthesis as a functional parameter in primary human myometrial cells has shown a complete block of PGE_2 release by

oxytocin when 5 μg/ml of troglitazone was added to the culture media minutes prior to oxytocin treatment (FIG. 2).

Examination of another functional parameter for oxytocin action, release of intracellular calcium from the endoplasmic reticulum into the cytoplasm of the cell, demonstrates that a 10 nM dose of oxytocin rapidly increases intracellular calcium release (FIG. 3A, each line represents calcium changes from a single cell). Without this increase in intracellular calcium within the myometrial cells, muscle contraction does not occur. In FIG. 3B, a 0.001 μg/ml of troglitazone (2.2 nM) has no effect upon the 10 nM stimulatory dose of oxytocin while in FIG 3C, a 0.01 μg/ml dose partially inhibits the oxytocin stimulated increase in intracellular calcium. In FIG. 3D, a 0.1 μg/ml dose of troglitazone completely inhibits the oxytocin stimulated increase in intracellular calcium. The effect of troglitazone is specific. 100 nM bombesin in the presence of 0.1 μg/ml troglitazone causes an increase in intracellular calcium levels (FIG. 3D). Bombesin is also a G_q coupled protein as is the OTR. Further evidence of specificity is demonstrated in FIG. 3E, FIG.3F, and FIG.3G in which bradykinin stimulation of intracellular calcium release is not inhibited by 1 μg/ml dose of troglitazone while that of 100 nM oxytocin stimulation is inhibited. A dose dependent increase in intracellular calcium is demonstrated in FIG. 3H in response to different concentrations of oxytocin (10^{-11} - 10^{-6} M, Control). A dose of 0.1 or 1 μM troglitazone demonstrates a dose dependent inhibition of oxytocin induced increases in intracellular calcium. Both doses of troglitazone shift the dose response curve to the right. At high levels of oxytocin in the presence of troglitazone (10^{-8} M OT/107 troglitazone and 10^{-7} M OT/10^{-6} M troglitazone), intracellular calcium levels are equivalent to those of OT-stimulation alone. This indicates that troglitazone is a competitive inhibitor as opposed to a noncompetitive inhibitor of oxytocin.

In FIG. 4, term myometrial tissue was obtained from C-section deliveries, and strips of tissue were suspended in a 37°C PBS bath under a low constant tension. This tension causes rhythmic contractions as demonstrated in FIG. 4A. 10 nM oxytocin causes a rapid increase in contractions that were sustained. After removal of oxytocin, the tissue reverts back to the rhythmic pattern of contraction. In FIG. 4B, a dose of 10 μg/ml of troglitazone inhibited 10 nM oxytocin induced contractions. A 100 nM dose

of oxytocin was able to overcome the troglitazone inhibition demonstrating reversibility of troglitazone treatment.

All of the compositions and/or methods disclosed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein (*e.g.*, other thiazolidinediones) while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

References

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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CLAIMS:

1. A method for preventing preterm labor in a pregnant subject comprising administering to the subject an amount of thiazolidinedione effective to prevent preterm labor in the subject.

2. A method for reducing an oxytocin-mediated action in a subject comprising administering to the subject an amount of thiazolidinedione effective to reduce the oxytocin-mediated action in the subject.

3. The method of claim 2, wherein the oxytocin-mediated action is induction of labor in a pregnant subject.

4. The method of claim 2, wherein the oxytocin-mediated action is induction of uterine cramps.

5. The method of claim 2, wherein the oxytocin-mediated action is induction of milk letdown.

6. The method of claim 2, wherein the oxytocin-mediated action is induction of prostaglandin release.

7. The method of claim 2, wherein the subject is a mammal.

8. The method of claim 2, wherein the thiazolidinedione comprises troglitazone.

9. The method of claim 2, wherein the thiazolidinedione comprises pioglitazone, BRL49653, or a compound related to troglitazone.

10. The method of claim 2, wherein the thiazolidinedione is dispersed in a pharmacologically acceptable form.

11. The method of claim 10, wherein said thiazolidinedione is administered locally.

12. The method of claim 10, wherein said thiazolidinedione is administered parenterally.

13. The method of claim 12, wherein said thiazolidinedione is administered intravenously.

14. The method of claim 11, wherein the thiazolidinedione is administered intravaginally.

15. The method of claim 3, further comprising administering a tocolytic agent.

16. The method of claim 15, wherein said tocolytic agent comprises a beta-mimetic, magnesium sulfate, a prostaglandin inhibitor, or a calcium-blocking agent.

17. The method of claim 16, wherein the prostaglandin inhibitor is indomethacin.

18. The method of claim 17, wherein the calcium-blocking agent is nifedipine.

19. The method of claim 15, wherein the tocolytic agent and thiazolidinedione are administered simultaneously.

20. A method of screening for an oxytocin agonist comprising administering a troglitazone-like compound to an oxytocin receptor and determining whether the compound binds the receptor.

21. A method of screening for an oxytocin antagonist comprising

(a) administering a thiazolidinedione to an oxytocin receptor;

(b) administering a composition comprising a candidate oxytocin antagonist; and,

(c) determining whether the thiazolidinedione binds to the receptor.

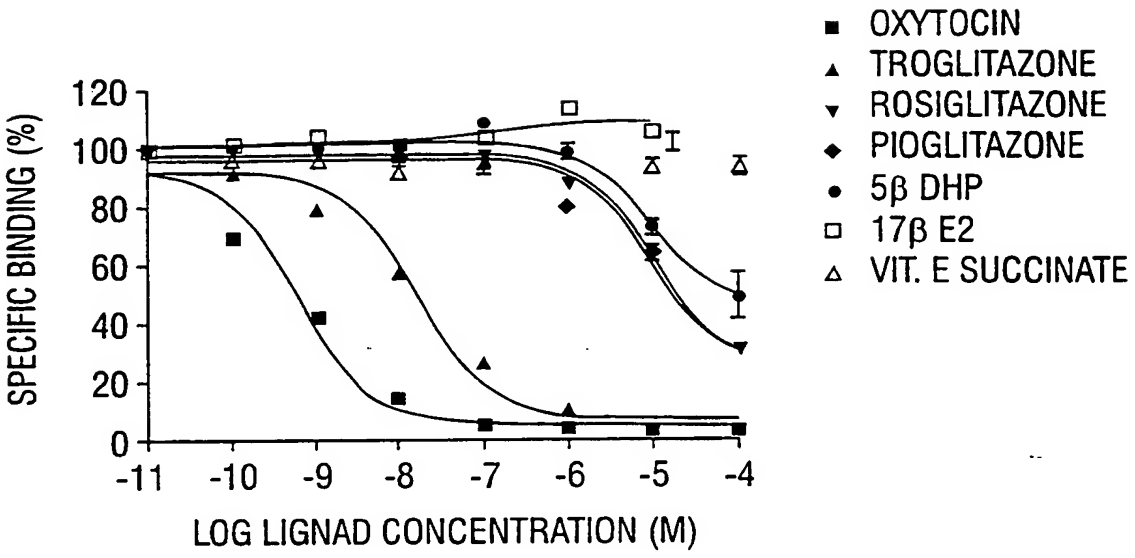
SPECIFIC BINDING (%)
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
-4
LOG LIGNAD CONCENTRATION (M)
OXYTOCIN
TROGLITAZONE
ROSIGLITAZONE
PIOGLITAZONE
5β DHP
17β E2
VIT. E SUCCINATE

FIG. 1

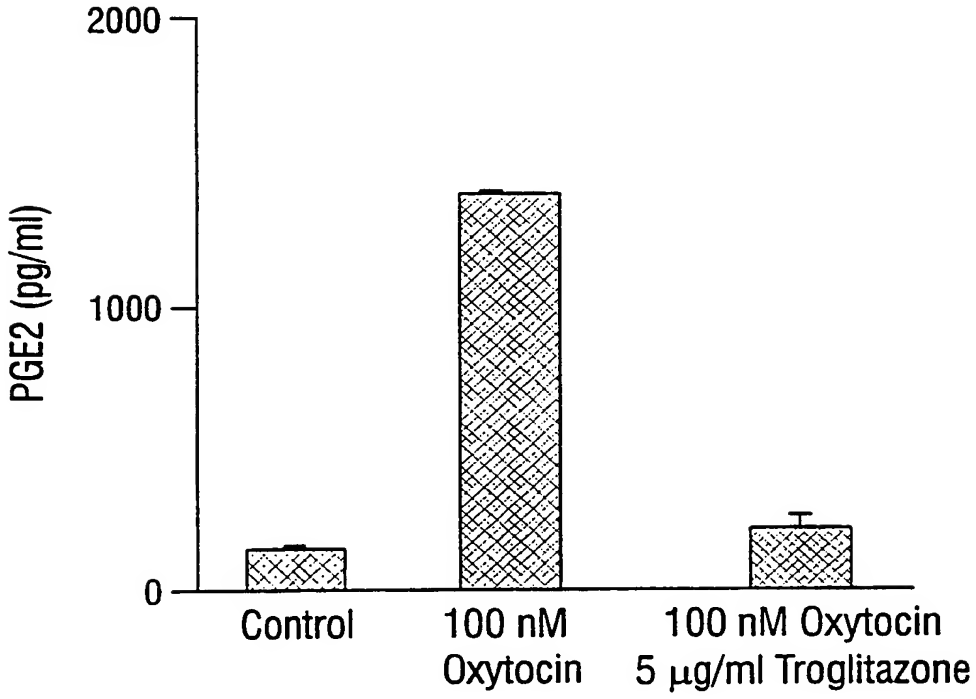
PGE2 (pg/ml)
2000
1000
0
Control
100 nM Oxytocin
100 nM Oxytocin 5 μg/ml Troglitazone

FIG. 2

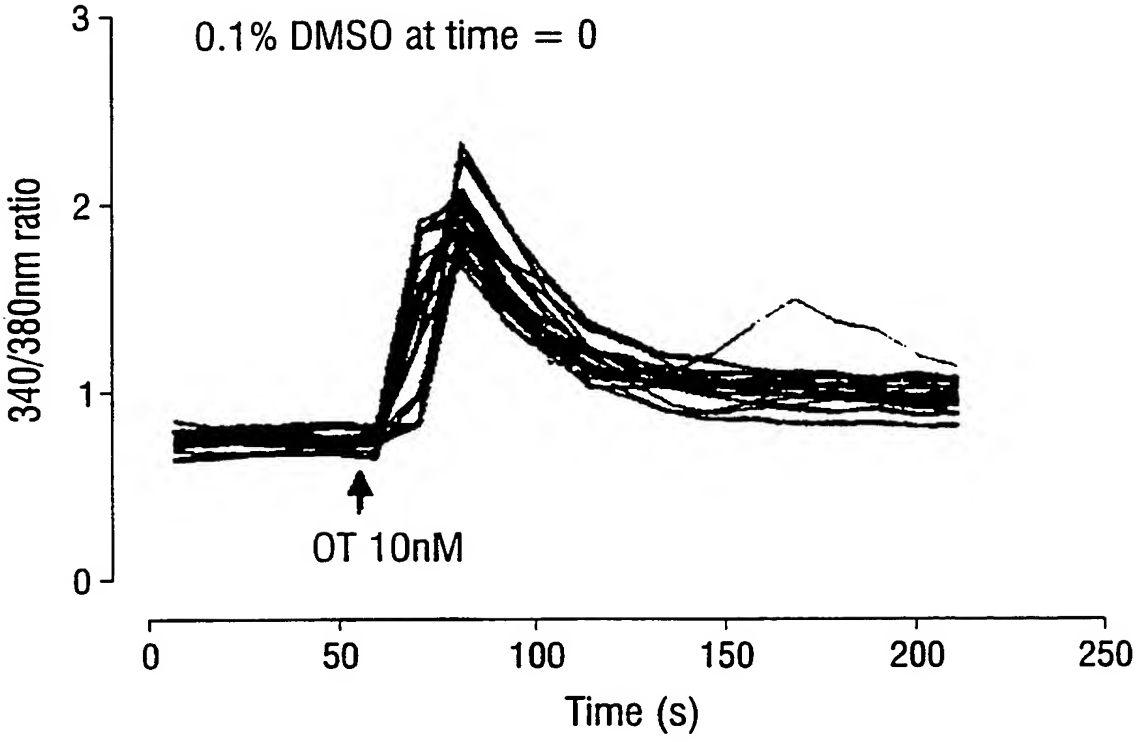
0.1% DMSO at time = 0
340/380nm ratio
3
2
1
0
OT 10nM
0
50
100
150
200
250
Time (s)

FIG. 3A

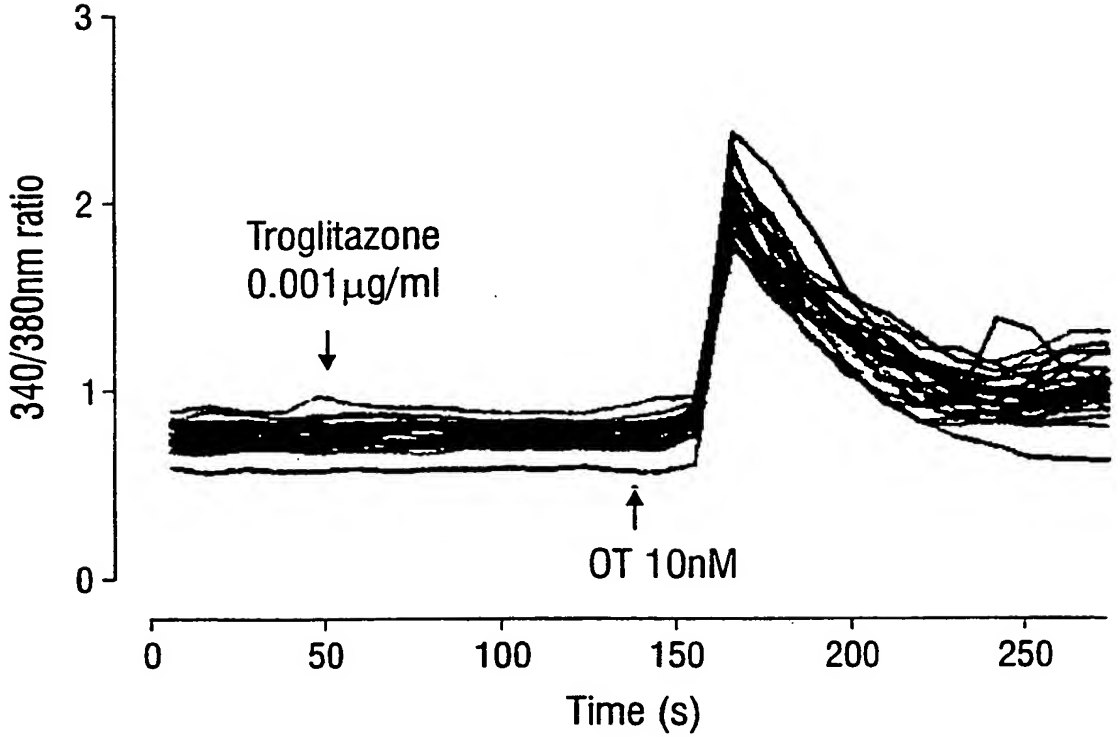
340/380nm ratio
3
2
1
0
Troglitazone
0.001µg/ml
OT 10nM
0
50
100
150
200
250
Time (s)

FIG. 3B

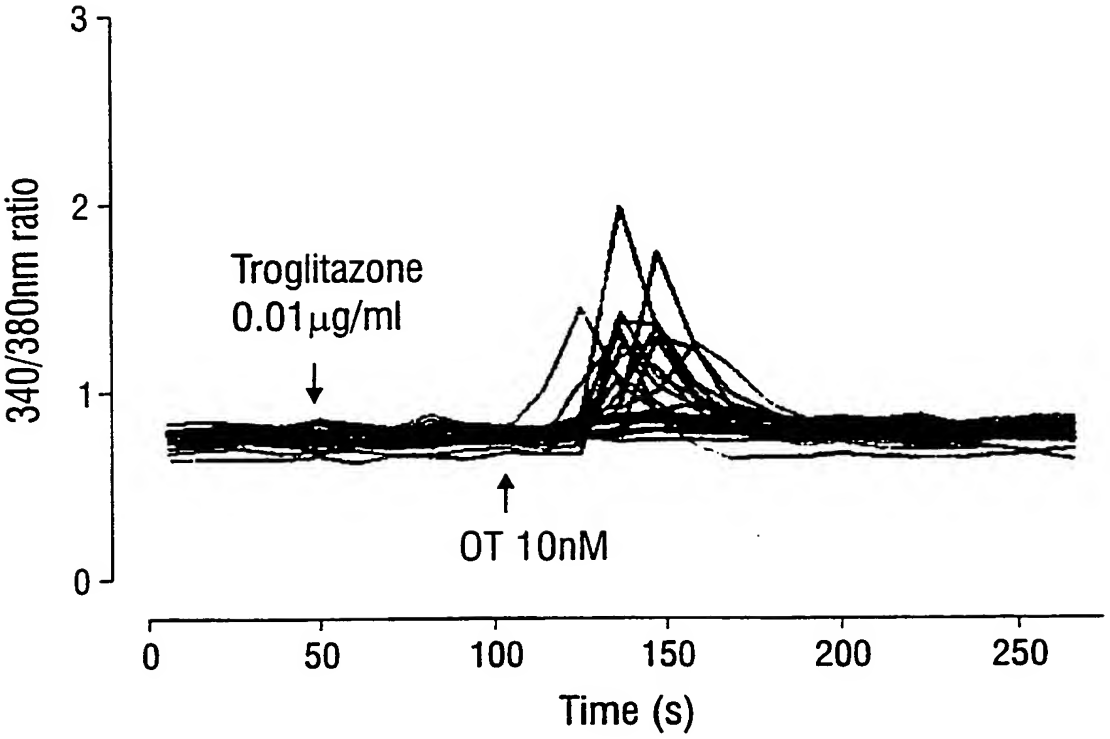
340/380nm ratio
3
2
1
0
Troglitazone
0.01μg/ml
OT 10nM
0
50
100
150
200
250
Time (s)

FIG. 3C

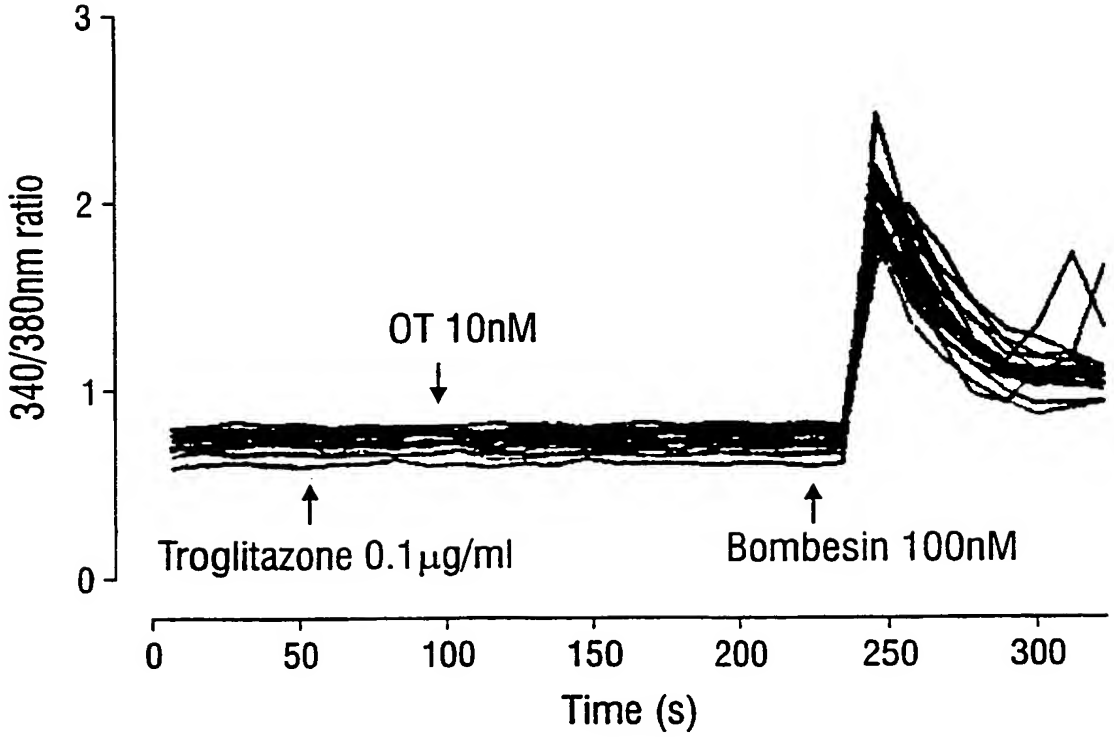
340/380nm ratio
3
2
1
0
OT 10nM
Troglitazone 0.1μg/ml
Bombesin 100nM
0
50
100
150
200
250
300
Time (s)

FIG. 3D

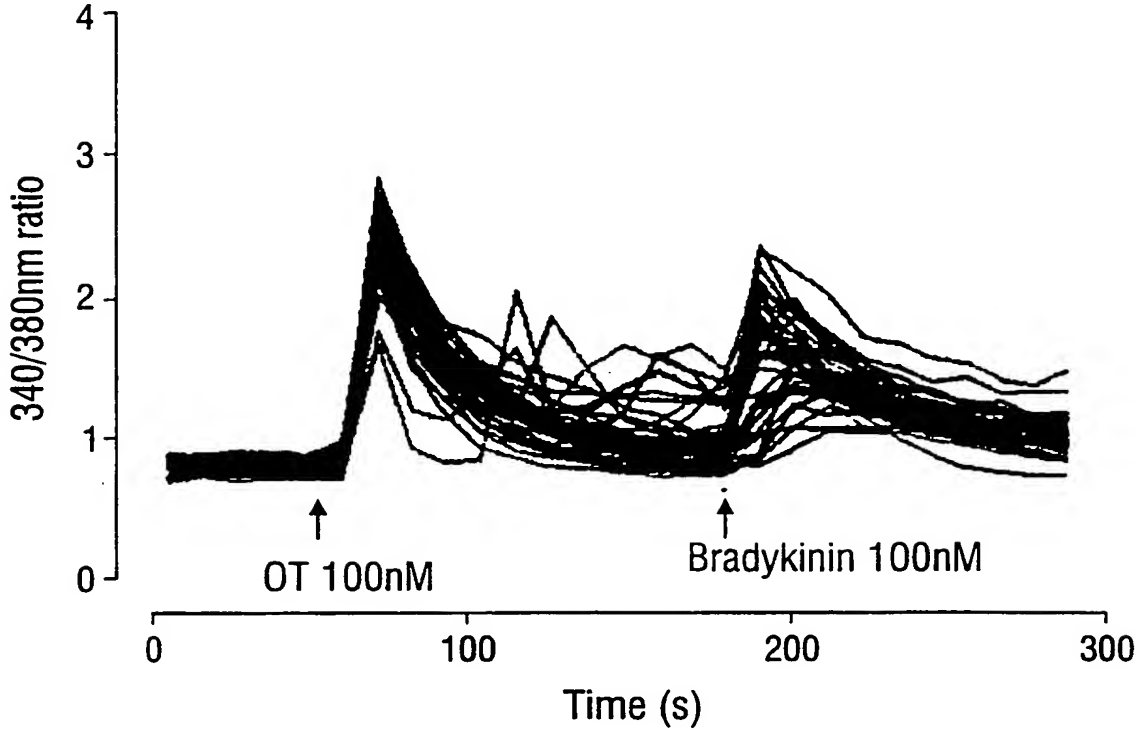
340/380nm ratio
4
3
2
1
0
OT 100nM
Bradykinin 100nM
0
100
200
300
Time (s)

FIG. 3E

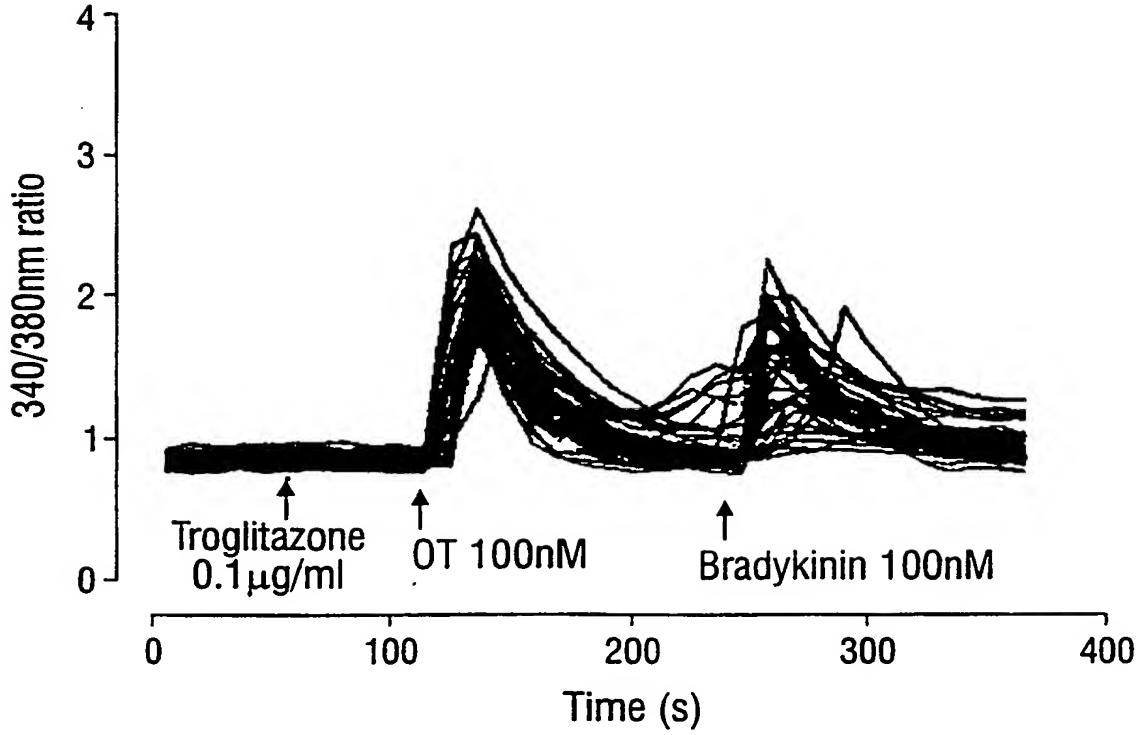
340/380nm ratio
4
3
2
1
0
Troglitazone
0.1μg/ml
OT 100nM
Bradykinin 100nM
0
100
200
300
400
Time (s)

FIG. 3F

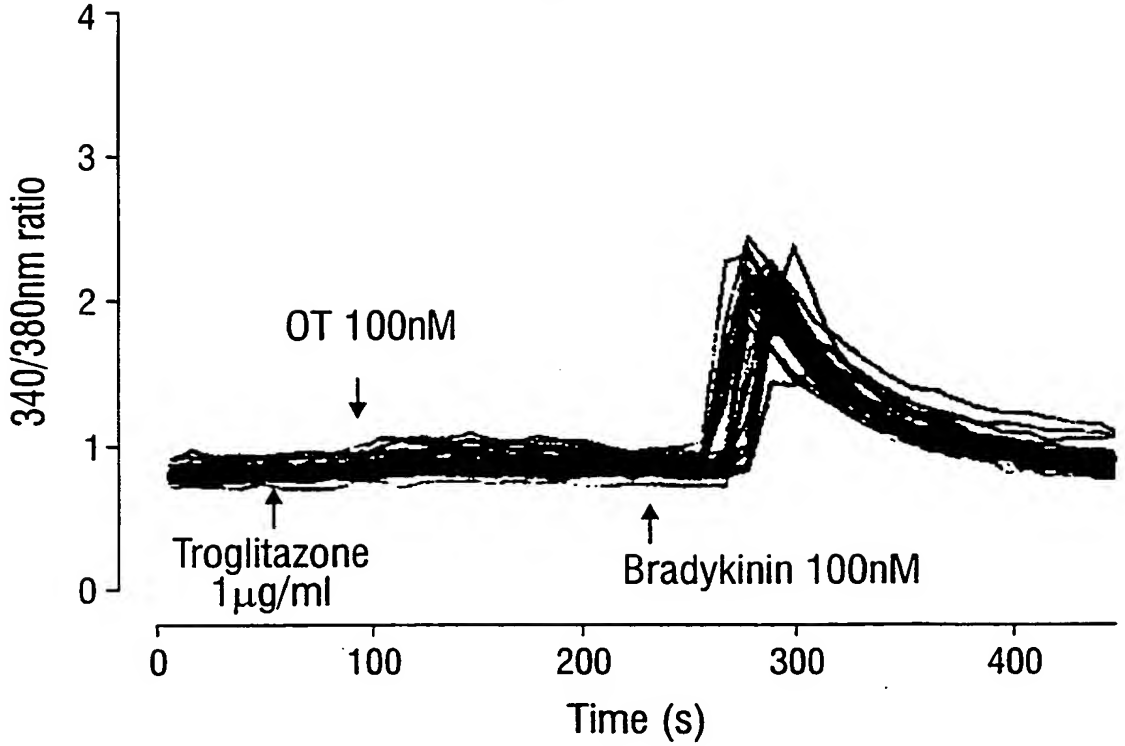
340/380nm ratio
4
3
2
1
0
OT 100nM
Troglitazone
1μg/ml
Bradykinin 100nM
0
100
200
300
400
Time (s)

FIG. 3G

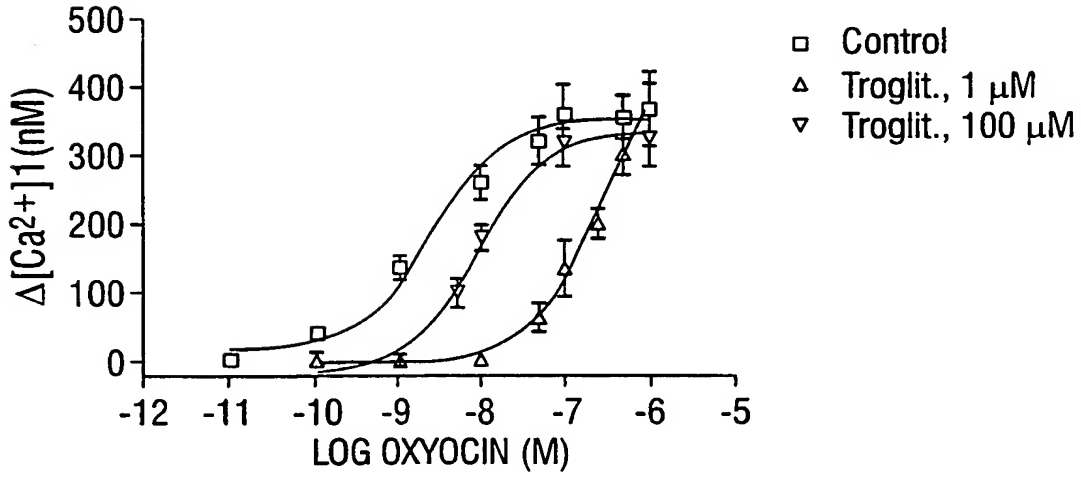
Δ[Ca2+]1(nM)
500
400
300
200
100
0
-12
-11
-10
-9
-8
-7
-6
-5
LOG OXYOCIN (M)
Control
Troglit., 1 μM
Troglit., 100 μM

FIG. 3H

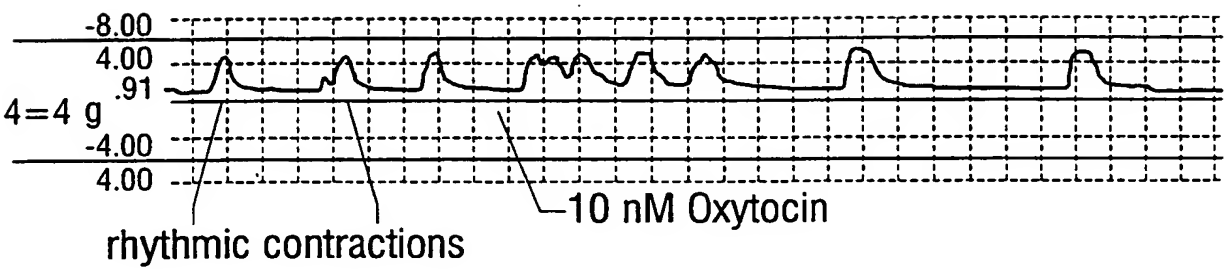
-8.00
4.00
.91
4=4 g
-4.00
4.00
rhythmic contractions
10 nM Oxytocin

FIG.4A

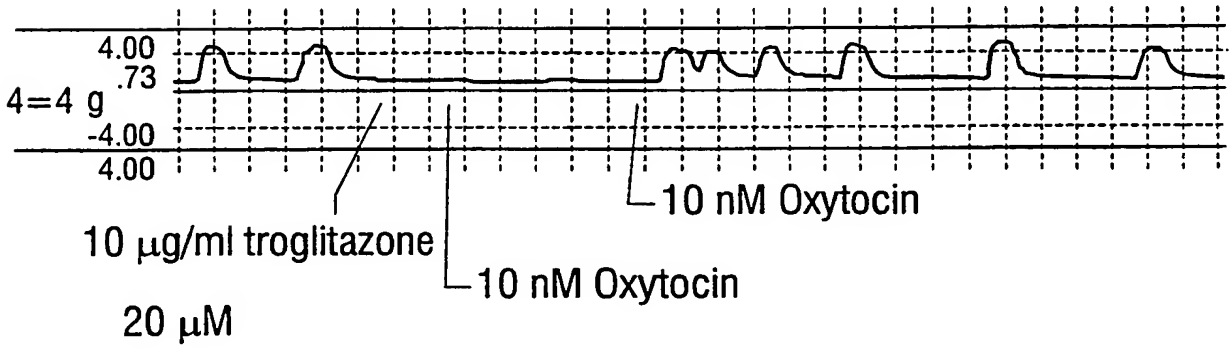
4.00
.73
4=4 g
-4.00
4.00
10 nM Oxytocin
10 μg/ml troglitazone
10 nM Oxytocin
20 μM

FIG4B

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 99/25433

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/427 A61K31/4439 A61P15/06 A61K31/426 G01N33/74

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 39006 A (GREEN ALLAN ;UNIV TEXAS (US); URBAN RANDALL J (US)) 11 September 1998 (1998-09-11) abstract examples 1-8 claims 1-24 page 4, line 1 - line 10	1-21
A	REECE E.A. ET AL: "Diabetes mellitus in pregnancy: What are the best treatment options?." DRUG SAFETY, (1998) 18/3 (209-220). , XP000889644 page 210, column 2, paragraph 2 page 215, column 2, paragraph 3	1-4,7,8, 10-14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
4 April 2000	10/04/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bonzano, C

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 861 666 A (TAKEDA CHEMICAL INDUSTRIES LTD) 2 September 1998 (1998-09-02) page 2, line 10 - line 33	1-21
A	EP 0 783 888 A (SANKYO CO) 16 July 1997 (1997-07-16) claims 1,2	1-21
A	WO 97 27191 A (REDDY S RESEARCH FOUNDATION DR ;REDDY CHEMINOR INC (US)) 31 July 1997 (1997-07-31) page 2 -page 3	1-21
A	CANCER RESEARCH, vol. 46, no. 4, 1986, pages 1735-40, XP000901165 abstract	1-21

INTERNATIONAL SEARCH REPORT

[redacted]ational application No.
PCT/US 99/ 25433

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 1-19
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 1-19 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: -
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
See FURTHER INFORMATION SHEET PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-7, 10-19 relate to an rather elevated number of possible compounds (thiazolidinediones). Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Furthermore, the expression "a compound related to troglitazone" is not clear, because it is not known how such relation should be interpreted in structural terms.

Present claims 15,16 relate to compounds defined by reference to a desirable characteristic or property, namely the activity as a tocolytic agent, as a beta-mimetic, as prostaglandin inhibitor or as a calcium blocking agent.

The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT for only a very limited number of such compounds. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the thiazolidindiones mentioned in claims 8,9, and to the compounds mentioned in claims 17-18, with due regard to the general idea underlying the present invention.

Claims searched completely: 8.
Claims searched incompletely: 1-7, 9-21

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 99/25433

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9839006 A	11-09-1998	US 5814647 A	29-09-1998
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XP-000901165

PD 04/1986
P 1735-1740 6

[CANCER RESEARCH 46, 1735-1740, April 1986]

Antitumor Efficacy in Rats of CGP 19984, a Thiazolidinedione Derivative That Inhibits Luteinizing Hormone Secretion[1]

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ABSTRACT

The antitumor efficacy and the hormonal effects of the thiazolidinedione derivative (sodium methyl{[3-methyl-2-[[5-methyl-3-(2-methylallyl)-4-oxo-2-thiazolidinyliden]hydrazono]-4-oxo-5-thiazolidinyl]}phosphate), CGP 19984, have been studied in *in vivo* rat prostatic and mammary cancer models. CGP 19984 significantly inhibited growth of the androgen-dependent Dunning R3327 rat prostate adenocarcinoma. Concomitant with tumor inhibition, a significant decrease in circulating luteinizing hormone and testosterone levels was observed, suggesting that the antitumor effects of drug treatment resulted primarily from inhibition of luteinizing hormone release and subsequently decreased testosterone synthesis. Drug treatment had little effect on serum prolactin or corticosterone levels. Animals showed no adverse effects from CGP 19984 except for a modest loss of body weight. In female rats, growth of the estrogen-independent MTW-9B rat mammary tumor was also inhibited by CGP 19984 and uterine weight and tumor progesterone receptor levels were reduced. The latter suggests that CGP 19984 treatment decreases circulating estrogen in female rats. However, the inhibitory effect of CGP 19984 on the growth of the MTW-9B tumor does not appear to be mediated by the action of the drug to lower estrogen levels, since this tumor is not dependent on estrogen for growth, and lower doses of CGP 19984 were found to be equally effective in reducing uterine weight, but had no antitumor activity. The ability of CGP 19984 to suppress gonadal function and to inhibit tumor growth suggests that this drug may have potential clinical application in the treatment of both hormone-dependent and -independent prostate and breast cancers.

INTRODUCTION

CGP 19984 is a derivative of thiazolidinedione (sodium methyl{[3-methyl-2-[[5-methyl-3-(2-methylallyl)-4-oxo-2-thiazolidinyliden]-hydrazono]-4-oxo-5-thiazolidinyl]}phosphate) (Fig. 1) which has been shown to cause regression of the hormone-dependent DMBA[3]-induced rat mammary tumor (2). In addition, moderate activity was demonstrated *in vivo* against a series of autonomous tumors, including the Walker 256 and colon 26 carcinomas, the R3230 AC mammary tumor [an estrogen-responsive, although estrogen-independent (3) tumor], and the Hardy-Passey melanoma (2). Inhibition of proliferation of several human cell lines *in vitro* has also been demonstrated (2). A predecessor drug to CGP 19984, the thiazolidinedione derivative GP 48989 (5-methyl-3-(2-methylallyl)-2-[(3-methyl-4-oxo-2-thiazolidinylidene)hydrazono]-4-thiazolidinone), was shown to inhibit the DMBA-induced mammary tumor, inducing regression of both estrogen-dependent (4) and estrogen-independent (5) forms of the tumor. In the former case it was suggested that GP 48989 caused tumor regression through an inhibition of gonadotropin release (6).

In view of the combined activity of these thiazolidinedione derivatives against both hormone-dependent and -independent tumors, the hypothesis was advanced that these derivatives might be effective agents in the treatment of breast and prostate cancers. Both types of cancer are heterogeneous in nature, and contain both hormone-dependent and -independent cell types. Relapse after endocrine therapy as the result of the outgrowth of hormone-independent cells, still remains a serious problem. Therefore, treatments which suppress growth of both hormone-dependent and -independent tumor cells should be more effective in inhibiting breast and prostate cancer than are treatments which act strictly as antihormonal agents. CGP 19984 was selected for further study because of its greater solubility than that of GP 48989.

The tumor models used in the experiments reported herein were the transplantable Dunning R3327 rat prostate adenocarcinoma and the MTW-9B rat mammary tumor. The former is a well-differentiated, slow-growing, androgen-dependent tumor that is histologically and histochemically similar to human prostate cancer (7, 8). It is composed of both hormone-dependent and -independent cells, with the former predominating, at least initially (7, 8). The tumor is responsive to castration (8), pharmacological doses of estrogens (8), the antiestrogen tamoxifen (9), as well as to LHRH agonists (10) and antagonists (11). The MTW-9B transplantable mammary tumor (12) grows in syngeneic Wistar-Furth rats. It is estrogen- and prolactin-independent, as evidenced by the lack of effect of ovariectomy or hypophysectomy on its growth rate. The MTW-9B tumor also does not respond to tamoxifen therapy (13). However, the estrogen receptor in the tumor appears to be functional since synthesis of the progesterone receptor is dependent on the presence of estrogen (14). The studies reported in this paper demonstrate the effectiveness of CGP 19984 in inhibiting growth of both the androgen-dependent R3327 prostate tumor and the estrogen-independent MTW-9B mammary tumor.

MATERIALS AND METHODS

Animals and Tumors. Male Copenhagen × Fischer F_1 rats bearing bilateral implants of the androgen-dependent well-differentiated R3327 Dunning rat prostate adenocarcinoma were kindly provided by Dr. Norman Altman of the Papanicolaou Cancer Research Institute in Miami, FL. Female Wistar-Furth rats were purchased from Harlan Sprague-Dawley (Indianapolis, IN), and were transplanted by trocar with the MTW-9B mammary tumor. Rats were housed in a temperature-controlled room with a 12-h light, 12-h dark, or 14-h light, 10-h dark schedule, and were fed rat chow (Teklad, Inc., Madison, WI) and water *ad libitum*.

Drug Preparation. CGP 19984 was supplied by CIBA-GEIGY, Ltd., Basel, Switzerland. It was dissolved in a vehicle of aqueous 0.5% carboxymethylcellulose (prostate experiments and mammary tumor experiment 3) or in aqueous 20% propylene glycol-0.5% carboxymethylcellulose (mammary tumor experiments 1 and 2). The drug was prepared fresh once or twice per week at concentrations of 5 or 50 mg/ml for the 25-mg/kg or 250-mg/kg dosage schedules, respectively. Each rat received 0.5 ml/100 g body weight of drug or vehicle, p.o., on a schedule of 5 times a week.

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[1] This work was supported in part by a grant from CIBA-GEIGY, Ltd., Basel, Switzerland. A preliminary report of this work was presented at the Thirteenth International Congress of Chemotherapy in Vienna (1).

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: LH, luteinizing hormone; LHRH, luteinizing hormone releasing hormone; DMBA, 7,12-dimethylbenz(*a*)anthracene; NIADDK, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases.

Table 1 *Effect of CGP 19984 treatment in intact male Copenhagen-Fischer rats bearing the R3327 prostate adenocarcinoma*[a]

	Treatment period (wk after transplant)	N[b]	Tumor wt[c] (g)	Seminal vesicle wt (g)	Serum testosterone (ng/ml)	Carcass wt (g)
Experiment 1						
Control	12–25	12	3.19 ± 0.70[d] (14)[e]	1.05 ± 0.09 (8)	3.36 ± 0.93 (8)	372 ± 12 (8)
CGP 19984		10	0.60 ± 0.15[f] (16)	0.23 ± 0.02[f] (8)	0.19 ± 0.05[f] (7)	307 ± 8[f] (8)
Experiment 2						
Control	13–26	10	26.3 ± 3.1 (16)	0.67 ± 0.04 (8)	1.51 ± 0.20 (8)	397 ± 12 (8)
CGP 19984		10	10.1 ± 1.3[f] (20)	0.46 ± 0.06[f] (10)	0.64 ± 0.08[f] (10)	390 ± 8 (10)
Experiment 3						
Control	14–25 and 18–29[g]	10	4.43 ± 1.22 (11)	0.70 ± 0.05 (8)	ND[h]	346 ± 9 (8)
CGP 19984		10	2.42 ± 0.75 (16)	0.22 ± 0.02[f] (9)	ND	309 ± 5[f] (9)
Experiment 4						
Control	27–38	11	2.45 ± 0.74 (19)	0.65 ± 0.04 (10)	1.94 ± 0.19 (10)	407 ± 7 (10)
CGP 19984		11	0.81 ± 0.12[f] (17)	0.24 ± 0.02[f] (9)	0.32 ± 0.07[f] (9)	329 ± 6[f] (9)

[a] The dose of CGP 19984 used in all these experiments was 250 mg/kg, p.o., 5 times a week.
[b] Number of rats per group at initiation of therapy.
[c] Rats were implanted bilaterally with the R3327 tumor. The tumor weight value represents the average weight of each tumor in the group.
[d] Mean ± SE.
[e] Numbers in parentheses, number of rats.
[f] Statistically different from corresponding control, $P < 0.05$.
[g] Rats were from 2 different transplant dates and were equally divided between the 2 groups.
[h] ND, not determined.

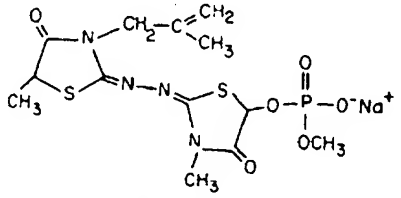

Fig. 1. Structure of CGP 19984. The chemical name of this drug is sodium methyl{[3-methyl-2-[[5-methyl-3-(2-methylallyl)-4-oxo-2-thiazolidinyliden]hydrazono]-4-oxo-5-thiazolidinyl]}phosphate. In some formulations, the sodium is replaced by ethanolammonium.

Experimental Protocol. Four experiments were done with intact rats bearing the R3327 prostate adenocarcinoma and one with prostate tumor-bearing rats castrated or sham castrated 1 day prior to initiation of CGP 19984 therapy. The R3327 tumor contains a heterogeneous cell population and shows a variable growth rate from transplant to transplant. The time at which CGP 19984 treatment was started in each of the 5 experiments was based on average tumor size rather than on time after transplant. In 4 of the experiments, treatment was initiated when the average tumor diameter was 3.5–5 mm; in the other (Table 1, experiment 2), average tumor diameter was 10 mm at start of therapy. The effects of CGP 19984 were tested in tumors of fast (Table 1, experiment 2), slow (Table 1, experiment 4), and average (Table 1, experiments 1 and 3) growth rates. In all prostate tumor experiments, the drug was administered at a dose of 250 mg/kg, p.o., until 24 h prior to sacrifice.

Three experiments were performed using rats bearing the MTW-9B transplantable mammary tumor. In these experiments CGP 19984 therapy was initiated either when the tumor was just palpable, or at the time of transplant (details are given in the legend to Fig. 8). CGP 19984 was tested at doses of 25 and 250 mg/kg, p.o. in these experiments, and was administered until 24 h prior to sacrifice.

In each of the experiments, tumor size was monitored by measuring tumor diameter in 2 perpendicular dimensions using a vernier caliper. At sacrifice, tumors and seminal vesicles or uteri were removed and weighed. Carcass weight was calculated as the difference between body weight and tumor weight for each animal.

Hormone Assays. The effect of CGP 19984 therapy on serum levels of testosterone, LH, prolactin, and corticosterone was determined at several time periods after initiation of therapy in castrated and sham-castrated rats bearing the prostate tumor. Blood was obtained by orbital sinus puncture under light ether anaesthesia at 0, 2, 4, 8, and 12 weeks, and after decapitation at 15 weeks. The effect of CGP 19984 therapy on serum testosterone was also determined in intact rats, from blood taken after decapitation. In all experiments, blood was collected between the hours of 9 and 11 a.m.

Serum testosterone levels were measured in duplicates of 100 μl using the method of Schwartz and Justo (15). Testosterone antiserum directed against testosterone-11-BSA (GDN No. 250) was kindly provided by Dr. G. D. Niswender of Colorado State University, and [1,2,6,7,16,17-^{3}H]testosterone (specific radioactivity, 135 Ci/mmol) was purchased from New England Nuclear (Boston, MA). Serum LH levels were measured in 50- and 100-μl aliquots using the NIADDK rat LH kit (National Hormone and Pituitary Program, Baltimore, MD) and were expressed as ng/ml in terms of NIADDK rat LH-RP-1. Serum prolactin levels were measured in duplicates of 20 μl by a standard radioimmunoassay method with a NIADDK rat prolactin kit, using the double antibody method of Niswender *et al.* (16). Serum prolactin levels are expressed as ng/ml in terms of NIADDK rat prolactin-RP-3. Serum corticosterone was measured using the procedure described by Henning (17), with [1,2,6,7-^{3}H]corticosterone (105 Ci/mmol) purchased from New England Nuclear. This assay is based on corticosterone binding to rat corticosteroid-binding globulin.

Receptor Assays. Mammary tumors were removed, weighed, and frozen in liquid nitrogen. They were stored at −80°F until receptor assay within 2 weeks. Steroid receptors were measured in the cytosol according to methods described previously (14, 18). Estradiol and dihydrotestosterone receptor were assayed using a single saturating concentration of 10 nM; progesterone receptor was assayed using a single saturating concentration of 20 nM. Protein in the cytosol was determined according to the procedure of Lowry *et al.* (19).

Statistics. The significance of the difference between two means was evaluated on the basis of Student's *t* test.

RESULTS

Effect of CGP 19984 on Growth of the Dunning R3327 Rat Prostate Adenocarcinoma in Intact Rats. The effect of CGP 19984 on growth of the prostate tumor was examined at a single dose of 250 mg/kg administered p.o., 5 times a week for 13 weeks. Growth of the tumor was markedly inhibited in animals treated with CGP 19984 (Fig. 2). At sacrifice, tumors from the treated group were only 19% of the size of those from the control group (Table 1, experiment 1). This inhibitory effect was confirmed in 3 additional experiments (Table 1, experiments 2–4) in tumors of varying growth rate. The range of tumor growth inhibition in all 4 experiments was between 45 and 81%.

No toxicity was apparent during treatment, except for some

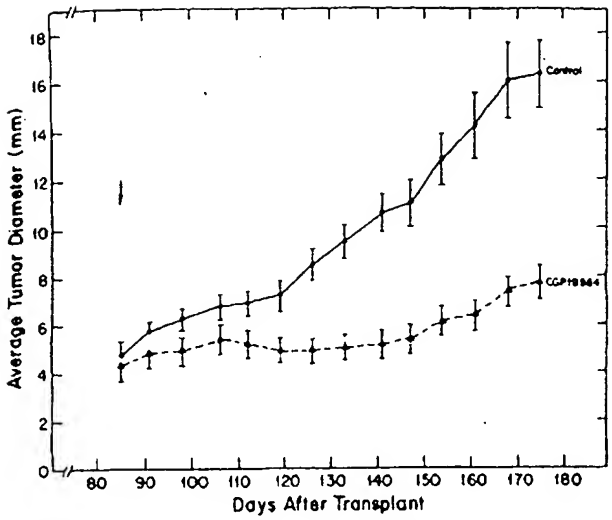

Fig. 2. Effect of CGP 19984 on growth of the R3327 rat prostate adenocarcinoma in intact male rats. This is experiment 1 of Table 1. *Points*, mean of 22 (control) and 20 (treated) tumors (initially) per group; *bars*, SE. The *arrow* indicates the time at which treatment was started. The curves are statistically different from each other from day 112 after transplant until the termination of the experiment ($P < 0.05$).

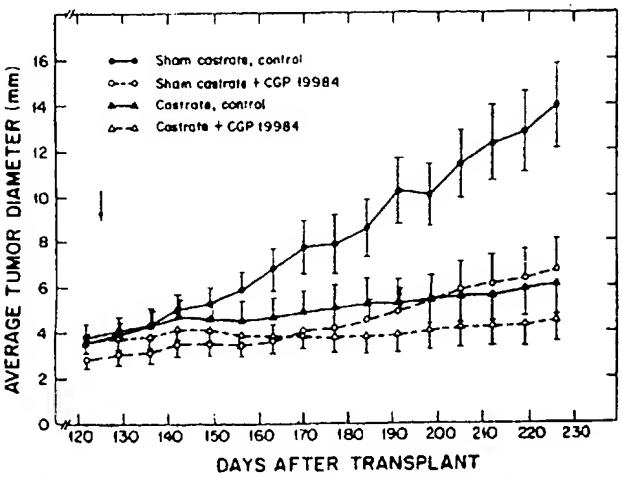

Fig. 3. Effect of CGP 19984 on growth of the R3327 rat prostate adenocarcinoma in sham-castrated and castrated male rats. Rats were sham castrated or castrated 124 days after transplant. Treatment was started the following day and continued for 15 weeks. *Points*, mean of 20 tumors (initially) per group; *bars*, SE. The *arrow* indicates the time at which treatment was started.

loss in body weight (Table 1) varying between 2% (experiment 3) and 19% (experiment 4). Serum testosterone concentrations were markedly reduced, with an inhibition ranging between 54 and 94% in 4 separate experiments. Reflecting this decrease, seminal vesicle weights were also decreased by CGP 19984 in all experiments. There was no decrease in levels of androgen receptor in the tumor upon CGP 19984 treatment, with concentrations of 63.8 ± 7.3 (SE) ($n = 12$) and 71.0 ± 5.7 ($n = 15$) fmol/mg protein in control and CGP 19984-treated groups, respectively (data from tumors of rats in experiment 4).

Effect of CGP 19984 on Growth of the Dunning R3327 Rat Prostate Adenocarcinoma in Castrated Rats. It has previously been demonstrated that 10–30% of the cells within the R3327 tumor are androgen independent, and as a result, relapse is eventually observed after castration (8). Since CGP 19984 has previously been shown to be active *in vitro* and effective against the hormone-independent R3230 AC mammary tumor *in vivo* (2), it was considered possible that CGP 19984 might have activity against both the androgen-dependent and -independent cell populations within the R3327 prostate tumor. To test this, rats bearing the R3327 tumor were castrated or sham castrated, and 1 day later treatment with CGP 19984 or vehicle was initiated. As can be seen in Fig. 3, both CGP 19984 and castration inhibited growth of the tumor. The combination of both appeared to be most efficacious. Although tumors in this group did not regress, growth did not exceed 25% over the 15 weeks of treatment. Statistically, however, the tumor growth curves of the castrated, castrated plus CGP 19984, and sham castrated plus CGP 19984 groups were not different.

Table 2 shows the effect of CGP 19984 on tumor weight as well as on the relative weights of various tissues and organs determined at necropsy. In the intact rat, the relative weights of the seminal vesicles and epididymus were significantly decreased by CGP 19984 treatment; the weights of the testes, thyroid, anterior pituitary, adrenals, thymus, and liver were not significantly different. In the castrated rat, thyroid and liver relative weights were increased by CGP 19984 treatment. Carcass weights were significantly decreased by CGP 19984 in both sham-castrated and castrated rats; however, the decrease in tumor weight in sham-castrated rats treated with CGP 19984 was still statistically significant when tumor weights were expressed per 100 g carcass weight. The body weights of the rats throughout the experiment are presented in Fig. 4.

Effect of CGP 19984 on Serum Concentrations of LH, Testosterone, Prolactin, and Corticosterone in Castrated and Sham-Castrated Male Rats. The effect of CGP 19984 on plasma hormone concentrations was determined in the castrated and

Table 2 *Effect of CGP 19984 in castrated and intact male Copenhagen-Fischer rats bearing the R3327 prostate adenocarcinoma*[a]

	Sham-castrated		Castrated	
	Control	CGP 19984	Control	CGP 19984
Tumor wt				
g	2.94 ± 0.76[b]	0.44 ± 0.25[c]	0.41 ± 0.22	0.39 ± 0.22
mg/100 g	799 ± 200	136 ± 77[c]	112 ± 58	123 ± 69
Carcass wt (g)	371 ± 16	321 ± 7[c]	353 ± 11	298 ± 8[c]
Seminal vesicle wt[d] (mg/100 g)	158 ± 6	103 ± 11[c]	29 ± 3	35 ± 3
Testes wt[d] (mg/100 g)	864 ± 19	873 ± 23		
Epididymus wt[d] (mg/100 g)	309 ± 15	255 ± 9[c]		
Thyroid wt[d] (mg/100 g)	8.9 ± 0.7	7.7 ± 0.5	7.4 ± 0.2	9.2 ± 0.6[c]
Anterior pituitary wt[d] (mg/100 g)	2.5 ± 0.2	2.9 ± 0.2	3.1 ± 0.3	3.1 ± 0.2
Adrenal wt[d] (mg/100 g)	16.1 ± 0.7	16.5 ± 0.7	15.5 ± 0.6	15.1 ± 0.9
Thymus wt[d] (mg/100 g)	28.8 ± 2.2	31.8 ± 3.7	48.1 ± 3.6	43.0 ± 4.6
Liver wt[d] (g/100 g)	3.2 ± 0.11	3.6 ± 0.2	2.6 ± 0.1	3.9 ± 0.1[c]

[a] CGP 19984 was administered at a dose of 250 mg/kg, p.o., 5 times a week.
[b] Mean ± SE. Sample size is 14, 9, 12, and 12 for the tumor weights in the sham-castrated control and CGP 19984-treated and castrated control and CGP 19984-treated groups, respectively. For all other weights, sample size is 7, 6, 6, and 6 for the 4 groups, respectively.
[c] Statistically different than corresponding control, $P < 0.05$.
[d] All tissue weights are expressed per 100 g carcass weight.

sham-castrated rats described above, from blood taken at 0, 2, 4, 8, 12, and 15 weeks after initiation of therapy.

Serum LH levels were significantly reduced in rats treated with CGP 19984 (Fig. 5). Two weeks after initiation of therapy, serum LH was decreased in sham-castrated rats to 11% of control values. LH levels remained suppressed in CGP 19984-treated rats for at least 1 month after the start of treatment, but thereafter there appeared to be a slow rise, with LH concentrations increasing to 38% of control at 8 weeks, 51% of control at 12 weeks, and 60% of control at 15 weeks. In castrated rats, LH concentration showed the characteristic increase in the weeks following castration to levels approximately 10-fold higher than those observed in intact rats. Strikingly, in castrated rats treated with CGP 19984, LH concentrations were reduced to 6–10% of the castrated control values for at least 3 months after initiation of therapy.

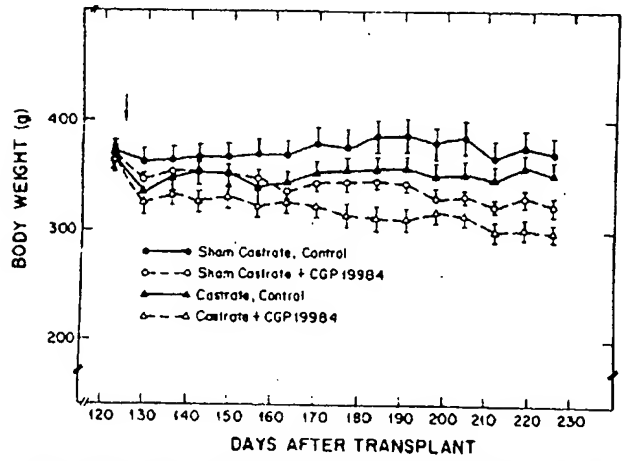

Fig. 4. Effect of CGP 19984 on body weight of sham-castrated and castrated rats bearing the R3327 rat prostate adenocarcinoma. This is from the same experiment described in the legend to Fig. 3. *Points*, mean of 10 rats (initially) per group; *bars*, SE. The *arrow* indicates the time at which treatment was started.

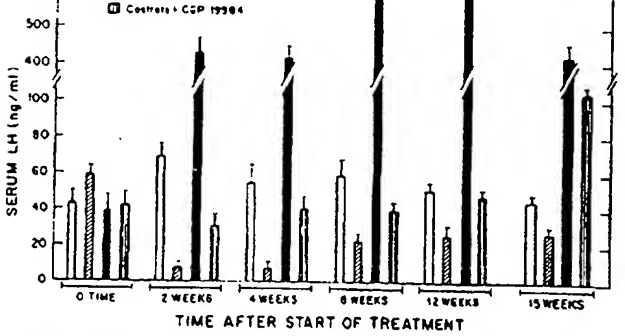

Fig. 5. Effect of CGP 19984 on serum LH concentrations in sham-castrated and castrated male rats at various times after initiation of therapy. Each *column* represents the mean of 6–10 rats per group; *bars*, SE. This is from the same experiment described in the legend to Fig. 3. The inhibitory effect of CGP 19984 on serum LH in both castrated and sham-castrated rats is statistically significant ($P < 0.01$) at all time points.

In response to the decreased levels of LH, serum testosterone levels also showed a marked reduction following CGP 19984 therapy (Table 3). Two weeks after the start of treatment, testosterone was reduced to 14% of control levels in intact rats, and at 4 and 8 weeks, the levels were below the minimum detectable in the assay. At later time periods, parallel to the increase of LH, testosterone concentrations began to rise again, reaching levels of 24 and 46% of control, respectively, at 12 and 15 weeks.

Figs. 6 and 7 show the concentrations of serum prolactin and corticosterone, respectively, at various times during treatment. Although there appear to be some changes at particular time points, no consistent pattern is evident with either hormone, and it appears as if CGP 19984 has no major effect on either prolactin or corticosterone.

Effect of CGP 19984 on Growth of the MTW-9B Mammary Tumor. Since previous data had suggested that CGP 19984 had cytotoxic or cytostatic activity (2), the drug was also tested *in vivo* against the MTW-9B rat mammary tumor. Fig. 8 shows that the 250-mg/kg dose of CGP 19984 partially inhibited the growth of the MTW-9B mammary tumor in 3 separate experiments. This inhibition was observed independent of the time of initiation of drug administration and did not appear to be a nonspecific effect resulting from loss of body weight (Table 4).

Interestingly, uterine weight was decreased by both doses of CGP 19984 (Table 4), suggesting that estrogen levels are reduced by this treatment. Further evidence for this is given by the fact that tumor progesterone receptor levels were reduced from 62.3 ± 10.3 fmol/mg protein ($n = 11$) in the control to 34.4 ± 10.5 ($n = 6$) and 26.7 ± 6.1 ($n = 9$) fmol/mg protein in the 25- and 250-mg/kg CGP 19984 treatment groups, respectively (Fig. 8, experiment 2). Tumor levels of estrogen and androgen receptor were measured, but were not significantly altered by either dose of CGP 19984 (data not shown).

DISCUSSION

The results presented here demonstrate that the orally active thiazolidinedione derivative, CGP 19984, effectively inhibits growth of the androgen-dependent R3327 rat prostate adenocarcinoma. Concomitant with this tumor inhibition, serum LH and testosterone concentrations were decreased, suggesting that CGP 19984 is acting via a chemical castration. The ability of CGP 19984 to decrease circulating testosterone concentration most likely results from inhibition of testosterone synthesis by the testes, secondary to a decreased circulating LH. It is unlikely that CGP 19984 affects the testes directly, since in this case LH levels would be elevated because of the loss of negative feedback, as is seen in castration. Additionally, a marked effect of CGP 19984 on serum LH was also noted in rats whose testes had been removed (Fig. 5).

Table 3 *Effect of CGP 19984 on serum testosterone concentration in intact and castrated Copenhagen-Fischer male rats at various times after initiation of therapy*

	Serum testosterone (ng/ml) at following times after start of treatment					
	0 wk	2 wk	4 wk	8 wk	12 wk	15 wk
Sham-castrate	2.40 ± 0.46[a]	1.51 ± 0.20	2.97 ± 0.92	1.87 ± 0.28	1.56 ± 0.31	2.26 ± 0.65
Sham-castrate + CGP 19984	1.86 ± 0.28	0.21 ± 0.05[b]	<0.1[b]	<0.1[b]	0.37 ± 0.14[b]	1.03 ± 0.41
Castrate	2.69 ± 0.54	<0.1	<0.1	<0.1	<0.1	<0.1
Castrate + CGP 19984	3.32 ± 0.76	<0.1	<0.1	<0.1	<0.1	<0.1

[a] Mean ± SE of 6–10 rats per group.
[b] Significantly different than sham-castrated control ($P < 0.01$).

Our results also demonstrate that CGP 19984 has moderate activity against the estrogen-independent MTW-9B rat mammary tumor. This finding, in conjunction with the report of Schieweck *et al.* (2) that the drug shows activity against a variety of hormone-independent tumors *in vivo* and of human cell lines *in vitro* suggests that CGP 19984 has cytostatic or cytotoxic activity as well as hormonal activity. Schieweck *et al.* (2) have also demonstrated that CGP 19984 shows marked antitumor activity against the estrogen-dependent DMBA-induced rat mammary tumor, an effect which is probably mediated by decreased ovarian estrogen synthesis secondary to decreased LH. That estrogen levels are in fact decreased after CGP 19984 therapy is supported by our finding that uterine weight and tumor progesterone receptor levels were decreased in treated rats. However, the inhibitory effects of CGP 19984 on the growth of the MTW-9B tumor do not appear to be mediated by the action of this drug to lower estrogen levels, since lower doses of CGP 19984 were found to be equally effective in reducing uterine weight, but had no antitumor activity. Moreover, the inhibition of growth of either the MTW-9B or R3327 tumors does not appear to be the result of inhibition of body growth, since body weight loss was relatively modest in comparison with the marked antitumor activity.

LHRH antagonists and agonists, chemically quite different from CGP 19984, have been shown to inhibit growth of both

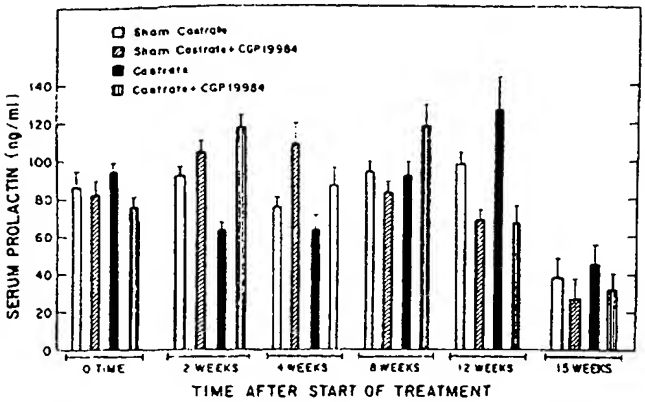

Fig. 6. Effect of CGP 19984 on serum prolactin concentrations in sham-castrated and castrated male rats at various times after initiation of therapy. Each *column* represents the mean of 6–10 rats per group; *bars*, SE. This is from the same experiment described in the legend to Fig. 3. In sham-castrated rats, the effect of CGP 19984 on serum prolactin is statistically significant at 4 weeks (increase) and at 12 weeks (decrease) ($P < 0.05$). In castrated rats, the effect of CGP 19984 is statistically significant at 2 weeks (increase) and at 12 weeks (decrease) ($P < 0.05$).

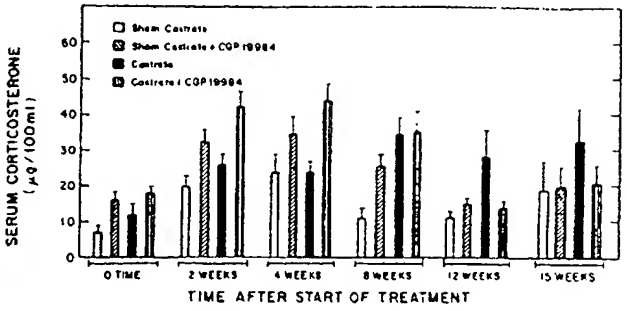

Fig. 7. Effect of CGP 19984 on serum corticosterone concentrations in sham-castrated and castrated male rats at various times after initiation of therapy. Each *column* represents the mean of 6–10 rats per group; *bars*, SE. This is from the same experiment described in the legend to Fig. 3. In sham-castrated rats, corticosterone levels in CGP 19984-treated rats are significantly higher than control at 0, 2, and 8 weeks ($P < 0.05$). In castrated rats, corticosterone levels in CGP 19984-treated rats are significantly higher than control at 2 and 4 weeks ($P < 0.05$).

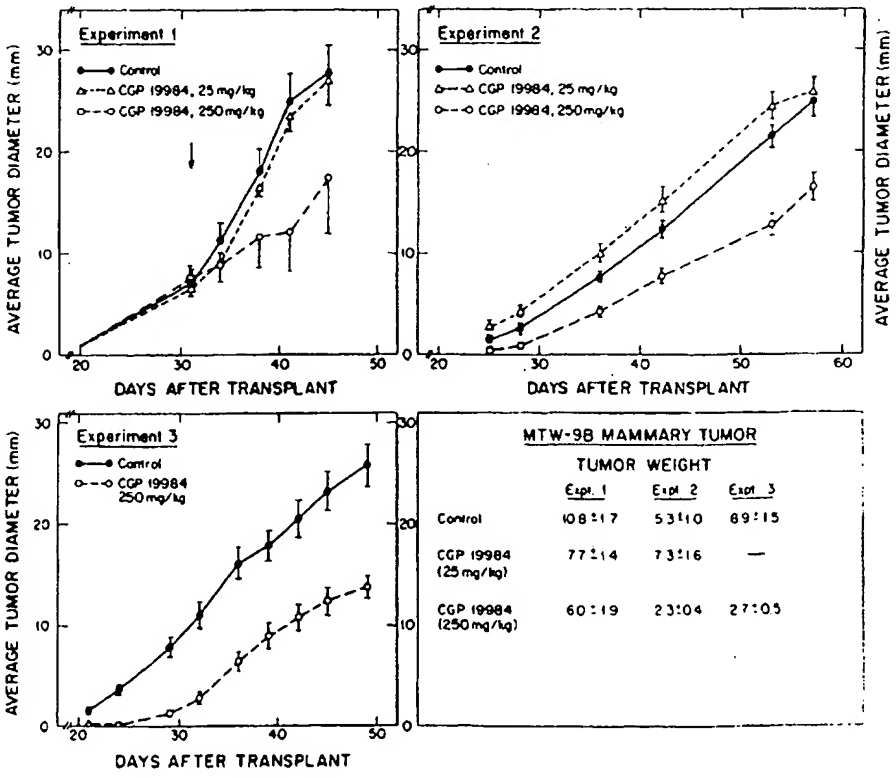

Fig. 8. Effect of CGP 19984 on growth of the MTW-9B mammary tumor in intact female rats. In experiment 1, rats were given injections of CGP 19984 or vehicle starting 31 days after transplant and continuing until sacrifice 45 days after transplant (10 injections total). The *arrow* indicates the time at which treatment was started. In experiment 2, rats were given injections of CGP 19984 or vehicle starting 2 days after transplant and continuing until sacrifice 57 days after transplant (40 injections total). In experiment 3, rats were given injections of CGP 19984 or vehicle starting on the day of transplant and continuing until sacrifice 49 days after transplant (35 injections total).

Table 4 *Effect of CGP 19984 on uterus and body weights of tumor-bearing female Wistar-Furth rats*

These are the same experiments described in the legend to Fig. 8.

Group	Uterine wt		Initial body wt[b] (g)	Final carcass wt (g)
	g	g/100 g[a]		
Experiment 1				
Control	0.36 ± 0.03[c]	0.20 ± 0.02	177 ± 5	184 ± 4
CGP 19984, 25 mg/kg	0.23 ± 0.02[d]	0.13 ± 0.01[d]	185 ± 4	183 ± 5
CGP 19984, 250 mg/kg	0.20 ± 0.02[d]	0.11 ± 0.01[d]	180 ± 5	182 ± 3
Experiment 2				
Control	0.39 ± 0.05	0.22 ± 0.03	172 ± 2	179 ± 3
CGP 19984, 25 mg/kg	0.19 ± 0.03[d]	0.11 ± 0.02[d]	172 ± 3	176 ± 3
CGP 19984, 250 mg/kg	0.18 ± 0.01[d]	0.10 ± 0.00[d]	177 ± 6	176 ± 4
Experiment 3				
Control	0.39 ± 0.03	0.21 ± 0.01	142 ± 3	185 ± 3
CGP 19984, 250 mg/kg	0.14 ± 0.01[d]	0.10 ± 0.01[d]	142 ± 2	144 ± 3

[a] Relative uterine weight (g/100 g carcass weight).
[b] Weight at start of therapy.
[c] Mean ± SE. There were 8–13 rats per group.
[d] Significantly different from corresponding control, $P < 0.01$.

the Dunning R3327 prostate tumor (10, 11) and a variety of estrogen-dependent mammary tumors (20). Currently, some of the agonists are in clinical trial, where at least in the case of prostate cancer, the results to date are highly encouraging (20). The more traditional therapy for disseminated prostate cancer, which has included castration, hypophysectomy, adrenalectomy, and diethylstilbestrol administration, has been reasonably successful in retarding disease (21). The limitations and disadvantages of ablative therapy are obvious, however, and estrogen administration, while avoiding the drastic surgical procedures, has undesirable side effects, the most severe of which is an increased incidence of cardiovascular disease. Inhibitors of LH release, which have been shown to be relatively nontoxic (20), appear to be superior to the above types of hormonal therapy.

The results reported herein suggest that the thiazolidinedione derivative CGP 19984 has potential clinical application in the treatment of prostate and breast cancers through its ability to decrease serum LH concentrations. Although the mechanism by which it suppresses LH is not yet understood, CGP 19984 appears to offer a distinct advantage over LHRH agonists and antagonists since, unlike the LHRH derivatives, it is orally active.

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